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AMERICAN SOCIETY OF ANDROLOGY

Seventh Annual Meeting and Postgraduate Course

February 23–26, 1982

Hyatt Hotel, Hilton Head Island, South Carolina, U.S.A.

Sponsored in Cooperation With:

The University of South Carolina School of Medicine

and

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SEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANDROLOGY

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Reception

A reception for the registrants and guests has been planned for Tuesday, February 23 from 7:00 to 9:00 PM in the Hyatt Ballroom. There will be a cash bar, with free hors d'oeuvres and free beer.

Poster Session

Approximately 64 abstracts will be presented in the poster session in the Hyatt Ballroom on Wednesday, February 24, from 6:30 to 8:30 PM. These abstracts include some of the highest rated abstracts of those submitted and cover a wide range of interests. Wine and cheese will be served without charge.

Workshop

A Workshop on "Practical Aspects of Laboratory Investigation" will be held on Wednesday, February 24, from 5:00 to 6:30 PM. Topics to be covered include: Technical Aspects of the *In Vitro* Bioassay of Gonadotropins, Determining Androgen and Estrogen Receptors in Prostate Cancer, and Technical Aspects of Estrogen Receptor Research. The panel members will be: M. Dufau, M.D., Ph.D., D. Coffey, Ph.D., and T. Abney, Ph.D. There is no fee for this workshop.

SEVENTH ANNUAL MEETING SCHEDULE

Postgraduate Course

Tuesday, February 23, 1982

Morning Session: The Male Accessory Glands—R. Lewis, M.D., *Chairperson*

8:00–8:50 AM	Prostatic Function: D. Coffey, Ph.D.
9:00–9:50 AM	Epididymal Function: S. Howards, M.D.
10:00–10:30 AM	Refreshments
10:30–11:20 AM	Seminal Vesicle Function: R. Eliasson, M.D.
11:30 AM–12:20 PM	The Role of Infection in Male Infertility: F. Derrick, M.D.
12:30–2:00 PM	Lunch

Afternoon Session: Receptors and Proteins in Testis Function—M. Dufau, M.D., Ph.D., *Chairperson*

2:00–2:45 PM	Physiologic Role of Estrogen in Testicular Development and Function: T. Abney, Ph.D.
2:45–3:30 PM	Isolation and Characterization of Gonadotropin Receptors: K. Catt, M.D., Ph.D.
3:30–4:00 PM	Refreshments
4:00–4:45 PM	Practical Aspects of Measuring Androgen Receptors and 5α-Reductase: J. Griffin, M.D.
4:45–5:30 PM	Chromosomal Proteins (Histones) and Germ Cells: S. Kistler, Ph.D.

Workshop

Wednesday, February 24, 1982

Tu Lin, M.D., *Chairperson*

5:00–5:20 PM	Technical Aspects of The *In Vitro* Bioassay of Gonadotropins: M. Dufau, M.D., Ph.D.
5:20–5:40 PM	Determining Androgen and Estrogen Receptors in Prostate Cancer: D. Coffey, Ph.D.
5:40–6:00 PM	Technical Aspects of Estrogen Receptor Research: T. Abney, Ph.D.
6:00–6:30 PM	Questions from Floor and Panel Discussion

Meet the Professor Luncheons

Wednesday, February 24, 1982

12:45–2:15 PM	The Varicocele and Infertility: F. Derrick, M.D.

Thursday, February 25, 1982

12:45–2:15 PM	The Semen Analysis: R. Eliasson, M.D.

Keynote Speakers

Wednesday, February 24, 1982

8:00–8:50 AM	Serono Award— K. Catt, M.D., Ph.D. (Regulation of Leydig Cell Function) M. Dufau, M.D., Ph.D. (Control of Androgen Biosynthesis and Secretion)

Thursday, February 25, 1982

8:00–8:50 AM	J. Griffin, M.D. (Normal Androgen Action and The Syndrome of Androgen Resistance)

Friday, February 26, 1982

8:00–8:50 AM	D. Coffey, Ph.D. (Prostate Carcinoma—1982)

Wednesday, February 24, 1982

8:00–8:50 AM	Plenary Lecture
9:00–10:30 AM	Session I—Testing Procedures: Abstracts 1–6
11:00–11:15 AM	Presidential Address
11:15 AM–12:45 PM	Session II—Biochemistry and Cell Biology: Abstracts 7–12
12:45–2:15 PM	Lunch "Meet The Professor" (optional)
Afternoon	Open
5:00–6:30 PM	Workshop
6:30–8:30 PM	Poster Presentations (Wine and Cheese) Testing: Abstracts 43–55 Biochemistry: Abstracts 56–66 Clinical Andrology A: Abstracts 67–72 Hormonal Regulation: Abstracts 73–80 Fertility Regulation: Abstracts 81–87 Physiology: Abstracts 88–97 Clinical Andrology B: Abstracts 98–106

Thursday, February 25, 1982

8:00–8:50 AM	Plenary Lecture
9:00–10:30 AM	Session III—Clinical Andrology A: Abstracts 13–18
11:00–11:15 AM	Awards Ceremony
11:15 AM–12:45 PM	Session IV—Hormonal Regulation and Drug Effects: Abstracts 19–24
12:45–2:15 PM	Lunch "Meet The Professor" (optional)
Afternoon	Open
5:00–6:30 PM	A.S.A. Business Meeting
7:00–8:00 PM	Cocktails (optional)
8:00 PM	Banquet "Country Bash" (optional) *Bring Your Best Levi's*

Friday, February 26, 1982

8:00–8:50 AM	Plenary Lecture
9:00–10:30 AM	Session V—Fertility Regulation: Abstracts 25–30
11:00 AM–12:30 PM	Session VI—Physiology, Development and Age-Related Studies: Abstracts 31–36
12:30–1:30 PM	Lunch
1:30–3:00 PM	Session VII—Clinical Andrology B: Abstracts 37–42
3:00 PM	Adjourn

INDEX TO AUTHORS OF ABSTRACTS

ABSTRACTS

SESSION I

Testing Procedures: Oral Presentations (1–6)

1. MIXED ANTIGLOBULIN REACTION AND SPERM CYTOTOXICITY TEST MEASURING ATP: TWO NEW METHODS FOR THE DETECTION AND TITRATION OF SPERMANTIBODIES. F. Comhaire and L. Vermeulen. *Department of Internal Medicine, State University Hospital, B-9000-GHENT-Belgium.*

Detection of spermantibodies using micro- or macro-agglutination techniques (described by Friberg and Kibrick respectively) is time consuming and results often are poorly reproducable. The Mixed Antiglobulin reaction (MAR-test, Jager et al, Int J Fertil 1978; p. 12) detects the presence of IgG class sperm antibodies on (motile) spermatozoa in the fresh ejaculate. This test is rapid, easy to perform and reproducable. In 80 patients the results of the MAR-test were compared with those of the Tray-agglutination test (Friberg) in semen plasma and in blood serum. The MAR-test detected spermantibodies only when the agglutinin titer in semen plasma or serum was "biologically significant" ie respectively $\geq$ 1/16 and $\geq$ 1/64. A positive MAR-test generally correlated with disturbed sperm penetration in cervical mucus. Cytotoxic antibodies in serum decrease the ATP-content of living spermatozoa when incubated in the presence of complement. ATP-measurement may be used to detect and titrate spermtoxic antibodies (Suominen et al, Arch Androl 1980; p. 257). The cytotoxicity test and Tray-agglutination test yielded similar results in 60 sera examined in parallel. The cytotoxicity test was easier to read because of the clear demarcation between positive and negative reactions and because of lack of non-specific positive reactions. It is advised to screen all semen samples for sperm antibodies by means of the MAR-test and to electively perform titration of spermantibodies in (male and/or female serum) using the ATP-sperm toxicity test. (This work was financed by core-support of the WHO Special Programme of Research, Development and Research Training in Human Reproduction).

2. EFFECT OF CYROPRESERVATION IN THE PRESENCE OF GLYCEROL ON MOTILITY AND FERTILIZING CAPACITY OF HUMAN SPERMATOZOA DETERMINED BY IN-VITRO HUMAN-HAMSTER TEST SYSTEM. Rajasingam S. Jeyendran, Hans H. Van der Ven, Mariano Perez-Pelaez, and Lourens J. D. Zaneveld. *Institute of Reproductive Medicine, Chicago, Illinois and University of Illinois, Chicago, Illinois.*

The aim of the study was to determine whether the cryopreserved sperm yields lowered fertility compared to fresh spermatozoa and if so, to ascertain the reason. Semen was frozen on dry ice into 0.1 ml pellets and stored for at least ½ h in liquid N_2 in all cryopreservation studies. Fertilizing capacity was determined by the ability of capacitated spermatozoa to penetrate denuded hamster oocytes. To determine the viability of cryopreserved spermatozoa, glycerol (10% v/v) was mixed with ½ of the semen and frozen. Cryopreserved sperm yielded significantly decreased ($P < 0.01$) motility and penetration into hamster oocytes compared to fresh spermatozoa. Even with equal numbers of cryopreserved and fresh motile sperm a significant reduction ($P < 0.01$) in penetration rate was noted. To determine whether the glycerol or the freeze-thawing procedure caused this reduction in penetration rate, pooled semen was divided and treated as follows: 1—no treatment, 2 and 3—mixed with glycerol, 4 and 5—glycerolated and frozen, and 6—frozen without glycerol. During capacitation and penetration procedures, portions 3 and 5 had glycerol treatment without subsequent freezing significantly decreased ($P < 0.05$) the penetration rate. However, no difference in penetration was noted when the glycerol treated sperm were allowed to penetrate the oocytes in the presence of glycerol as compared to fresh spermatozoa or between sperm cryopreserved with and without the addition of glycerol. A significantly higher ($P < 0.05$) penetration rate was noted when the glycerolated frozen spermatozoa were allowed to penetrate into oocytes in the presence of glycerol. Thus, glycerol, although beneficial for cryosurvival, its partially responsible for the reduced sperm motility and penetration into hamster oocytes.

3. A COMPARISON OF THE PAPANICOLAOU AND BURSTONE METHODS FOR THE EVALUATION OF THE MORPHOLOGY AND CYTOLOGY OF CELLS IN HUMAN SEMEN. Margaret L. Couture and Matthew Freund. *Department of Obstetrics and Gynecology, New Jersey School of Osteopathic Medicine, Camden, New Jersey.*

Currently, semen analysis includes morphological classification of cells into 3 populations: spermatozoa, immature germ cells (IGC), white blood cells. This practice does not take into account the exfoliated genitourinary tract cells (EGTC) in human semen. A study was made

to determine the types and quantitate the number of EGTC in 59 specimens from 24 vasectomized men (30–73 years old) and 54 specimens from 21 nonvasectomized men (20–58 years old). Vasectomized men were used to eliminate the possibility of confusing EGTC with IGC. Each specimen was divided into two aliquots, processed, and stained either by standard Pap technique or by Burstone method (to detect acid phosphatase activity in exfoliated prostate cells). Mean values for EGTC from vasectomized men were: prostate cells, 466/ml (250–2456); urothelial cells (from renal pelvis, ureter, or bladder), 862/ml (22–1211); squamous cells from ureter, 235/ml (92–349); seminal vesicle cells, 279/ml (90–524); and multinucleate cells of undetermined origin, 16/ml (4–28). The most prevalent type of white blood cell was the neutrophil, 6670/ml (1352–11404); macrophages and lymphocytes were found in small numbers, 60/ml (22–128). Identification of EGTC was made in vasectomized specimens by both Pap and Burstone methods. Comparison of Pap and Burstone methods in nonvasectomized specimens showed that after Pap staining, it was difficult to distinguish between exfoliated prostate cells and IGC (especially early spermatids and secondary spermatocytes) or lymphocytes. However, these were distinguishable after use of the Burstone method because of its specificity for prostatic acid phosphatase activity, present in high concentration only in prostate cells. These data call for a reevaluation of the current technique of morphological classification of semen, since cells classified as IGC may not have been recognized as EGTC.

4. REACTION OF THE HUMAN SPERM MEMBRANE TO HYPOOSMOTIC STRESS—RELATIONSHIP TO FERTILITY H. H. van der Ven, R. S. Jeyendran, M. Perez-Pelaez, L. J. D. Zaneveld, and B. G. Crabo. *Institute of Reproductive Medicine, Chicago, Illinois, University of Illinois, Chicago, Illinois, and University of Minnesota, St. Paul, Minnesota.*

The percentage of spermatozoa with normal membranes as indicated by swelling of the sperm tails after incubation of sperm in a hypoosmotic solution (Drevius and Eriksson. Exp Cell Res 1966; 42:136), was determined in a modified hemocytometer with phase contrast microscopy. Solutions tested contained melitose, sucrose, fructose, Na-citrate and/or NaCl (n = 35). A mixture of equal parts of fructose and Na-citrate (150 mOsm) was optimal in producing a clearly identifiable swelling of a maximum number of spermatozoa. Twelve to 15 determinations of the percentage of spermatozoa in three ejaculates of semen resulted in a SD of 5.5, 4.2, and 2.6 with a mean of 35.3, 65.2, and 80.9 respectively. When known numbers of spermatozoa, killed by incubation at 56 C for 30 min were added to fresh semen, the correlation between expected and observed values of swollen spermatozoa was 0.94 (n = 12). When the percentage of spermatozoa that showed swelling under hypoosmotic stress and the percentage of denuded hamster oocytes penetrated by the spermatozoa from the same semen were tested, a correlation of 0.83 was observed (n = 16). By contrast, little correlation (0.21) was found between the percent abnormal sperm forms in the ejaculate and the percent swollen spermatozoa while a somewhat higher correlation (0.60) was found between the percent sperm motility and the percent swollen spermatozoa (n = 21). This assay technique to evaluate the functional integrity of the sperm membrane by subjecting the sperm to hyoosmotic stress, gives high repeatability, consistency, and is closely correlated to the *in vitro* fertilizing capacity of spermatozoa. It appears to be a worthwhile addition to the standard semen analysis. (In part supported by NIH HD 09868).

5. INHIBITION OF COMPLEMENT-MEDIATED HEMOLYSIS BY AQUEOUS EXTRACTS OF MONKEY PROSTATE AND SEMINAL VESICLE. Thomas H. Tarter and Mohamed Isahakia. *Oregon Regional Primate Research Center, Beaverton, Oregon.*

Human seminal plasma (HSP) has been shown to inhibit complement-mediated hemolysis (J Lab Clin Med 96:582, 1980). To identify the anatomical origin of a seminal plasma complement inhibitor, we tested aqueous extracts of prostate (PE) and seminal vesicle (SVE) of the cynomolgus macaque (*Macaca fascicularis*) in a standard immune hemolytic assay. The protein content of the extracts was adjusted to 20 mg/ml. Bovine serum albumin (BSA) and HSP (each 20 mg of protein/ml) were used as negative and positive controls, respectively. Serial dilutions ranging from 1:10 to 1:640 were tested. At a 1:10 dilution, HSP inhibited hemolysis by 30%, PE by 82%, and SVE by 55%. At a 1:40 dilution, PE inhibited by 28% and SVE by 24%. The BSA had no effect on complement-mediated hemolysis. When antibody-coated erythrocytes were incubated with undiluted PE and SVE, washed, and used in the hemolytic assay, no inhibition was observed, an indication that the antihemolytic factor is complement-specific. The PE and SVE were passed through a Sephadex G-200 column, and maximum antihemolytic activity eluted at the same volume for both extracts. Comparing this volume with the elution volumes of several marker proteins, we estimate the molecular weight of the seminal plasma complement inhibitor to be 60,000. (Supported by Public Health Service Training Grant 1-T32-HD-0-7133-03).

6. SEVERAL VARIATIONS OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (EIA) TO DETECT ANTIBODY TO HUMAN SPERM. Steven B. Ackerman and J. W. E. Wortham, Jr. *Andrology Laboratory, Department of Biological Sciences, Old Dominion University, Norfolk, Virginia.*

Recently, we reported an EIA method to detect and quantitate antibodies to human sperm by adsorbing whole sperm to wells of polystyrene microtiter plates (Ackerman et al, Am J Reprod Immunol 1981; 1:199).

Comparison of this method to commonly performed agglutination and immobilization assays indicated that EIA was easier to perform, more sensitive and less subjective in interpretation of results. Several variations of this EIA method have been devised employing soluble extracts (saline and detergent) adsorbed to microtiter wells and intact sperm in suspension. These variations extend the applicability of EIA to research and diagnostic functions in antisperm antibody studies.

SESSION II

Biochemistry and Cell Biology: Oral Presentations (7–12)

7. LOCATION OF SURFACE ANTIGENS FROM PLASMA MEMBRANES (PM) AND PM POLYPEPTIDES OF BOAR SPERMATOZOA. L. D. Russell, T. A. Russell, and R. N. Peterson. *Department of Physiology and School of Medicine, Southern Illinois University, Carbondale, Illinois.*

Surface location of boar sperm antigens capable of blocking sperm-egg binding was determined using four different labelling techniques. Fluorescence labelling, using FITC conjugated IgG showed antigens were present primarily over the head PM and mid-piece PM of the flagellum. Fluorescence in the head was most prominent over the postacrosomal region. This localization was confirmed ultrastructurally by indirect ferritin and peroxidase techniques. IgG binding sites were also located directly by TEM by a direct visualization method which takes advantage of the sharp definition of PM obtained by postfixing with a ferrocyanide-osmium mixture (Russell and Burguet, 1977). IgG is seen as a fuzzy coat that extends laterally about 12 nm from surface binding sites. The pattern of antibody binding sites obtained by this direct method is similar to that obtained by the indirect methods. These labelling methods are now being applied to the localization of specific PM polypeptides. In initial trials, PM was fractionated by preparative SDS-PAGE. Five groups of polypeptides (the major Coomassie staining proteins) were cut from the gels and the gels were used to immunize rabbits. Distinct FITC patterns on fixed sperm were obtained for each polypeptide group. Fluorescence was confined to the postacrosomal region for one group of acidic glycopolypeptides with apparent molecular weight near 39K. IgG to a second group of low molecular weight polypeptides, some of which are thought to be concentrated in the principal segment of the head, produced a diffuse fluorescence pattern over the entire sperm. This was the only PM fractionated antiserum, however, to elicit any significant fluorescence over the principal segment. These experiments provide a methodological basis for fractionating and determining the location of the major surface antigens of boar spermatozoa. (Supported by NIH 14897).

8. REFINEMENT OF HUMAN PROSTATE ANDROGEN RECEPTOR QUANTITATION: EFFECTS OF TEMPERATURE AND MOLYBDATE/DITHIOTHREITOL. Robert W. Boesel, Robert W. Klipper, and Sydney A. Shain. *Southwest Foundation for Research and Education, San Antonio, Texas.*

Addition of 20 mM molybdate (M) and 20 mM dithiothreitol (DTT) to cytosol did not alter total cytoplasmic androgen receptor (R_cT) detected by incubation with R1881 (2.5–110 nM) for 20–24 hr at 15° (120 ± 24% of control, mean ± SD). M and DTT increased the apparent affinity (K_{R1881}) of R1881 for R_cT 2–3 fold, improving assay sensitivity. In the presence of M and DTT, R_cT values obtained by incubation at 2° and 15° were significantly different ($P < 0.05$) for 16 of 24 specimens. R_cT was greater at 15° in 11 of these specimens. Four specimens gave equivalent, non-zero ($P < 0.05$) values for R_cT at 2° and 15°. The remaining four specimens gave values for R_cT at 2° and 15° which were not significantly different from zero ($P < 0.05$). Although paired analyses indicated significant differences in R_cT determined at 2° and 15°, the pooled R_cT data was highly correlated ($r = 0.85$) and indicated equivalency for R_cT quantitated at 2° and 15°. K_{R1881} values at 15° were 2–3 times lower than those obtained at 2°. Inclusion of M and DTT in the homogenization buffer caused significant reduction of total nuclear androgen receptors (R_nT) detectable in nuclear KCl extracts. This was avoided by adding M and DTT to cytosol after separation from the nuclear pellet. R_nT of six prostate specimens was quantitated by incubation of nuclear KCl extracts with R1881 (0.5–10 nM) for 20–24 hr at 2° and 15°. Mean R_nT values were identical for both temperatures; however, R_nT determined at 15° was significantly greater ($P < 0.05$) for two specimens. Mean K_{R1881} for R_nT measured at 15° was 30% of that at 2°. Our data demonstrate improved R_cT quantitation in the presence of M and DTT. Incubation at 15° is indicated for maximum R_cT and R_nT detection. However, the reduction in K_{R1881} at 15° suggests diminished assay sensitivity.

9. PROTEIN KINASE FROM DISPERSED CANINE LEYDIG CELLS. Richard N. Anderson. *Departments of Obstetrics/Gynecology and Biochemistry, University of Tennessee College of Medicine, Memphis, Tennessee.*

Dispersed Leydig cells from canine testes were prepared by collagenase (0.5 mg/ml) treatment in medium 199. Protein kinase activity from these dispersed cell preparations has been partially characterized. With histone type II A (Sigma) as substrate, the optimal ATP conc. is 300 μM with 2.84 mM NaF and 0.91 to 14.5 μM cAMP. In comparing substrates, a lysine-rich histone from calf thymus (type 1, Johns EW, Biochem J 1964; 92:55; or type V-S, Sigma) was found to accept three times more phosphate than histone type IIA at equal conc. Protein kinase activity with either substrate was inhibited by

NaCl and KCl in a dose-dependent manner. In the absence of salt, a casein protein kinase activity was stimulated 2 fold by cAMP; in the presence of 0.3 M KCl, the casein protein kinase was stimulated 3 fold (relative to no KCl) and was not further stimulated by cAMP. CMC chromatography of Type 1 histone yielded two major fractions with phosphate acceptor activity and 4 minor fractions with little or no acceptor activity. The two major fractions each contain a major band and minor components by PAGE at pH 6.6. The acceptor activity appears to be associated with the prominent bands on PAGE. Attempts at isolating these active substrates are in progress. Since histones are highly conserved structures, the natural substrate(s) of dispersed canine Leydig cells may be closely related to these lysine-rich histones.

10. FURTHER PURIFICATION OF ANDROGEN RECEPTOR FROM STEER SEMINAL VESICLE. Donald J. Tindall, Ching H. Chang, and David R. Rowley. *Baylor College of Medicine, Houston, Texas.*

We have reported previously the purification of the androgen receptor from steer seminal vesicle by affinity chromatography (Fed Proc, 1980; 39:2134). We now report a new procedure which allows purification to apparent homogeneity. Two techniques were utilized—differential DNA-chromatography and steroid affinity chromatography. Cytosol (105,000 × g) was prepared in 50 mM Tris-HCl buffer containing 1.5 mM EDTA, 1.5 mM dithiothreitol, 20% glycerol (TEDG buffer) and 10 ug/ml leupeptin—a protease inhibitor. Cytosol was passed through the first DNA-Sepharose column, and flow-through fractions were precipitated with 40% ammonium sulfate. The precipitate was resuspended in TEDG buffer and applied to testosterone-hemisuccinyl diaminodipropylamine Sepharose 4B. The receptor was eluted with 100 ug/ml testosterone in TEDG buffer. The eluate was applied to a second DNA-Sepharose column and eluted with 10 mM pyridoxal-5'-phosphate in sodium borate buffer (pH 8.1). The procedure yields about 6 ug of receptor from 35 g of steer seminal vesicle, with a recovery of 15% and 82,000-fold purification. A single band of 60,000 daltons was observed following electrophoresis in SDS-polyacrylamide. The receptor protein had an estimated Stokes radius of 35 Å and a sedimentation coefficient of 3.8S in 0.3 M NaCl, indicating a molecular weight of 60,000 and a frictional ratio of 1.42. Chromatofocusing of the purified protein revealed an isoelectric point of 6.6 [^{3}H]Methyltrienolone was displaced from the purified protein with methyltrienolone > testosterone > 5 α-dihydrotestosterone >> dehydroepiandrosterone >> 5 α-androstane-3, 17 β diol. The physicochemical properties of the purified receptor are similar to those of the receptor in crude cytosol. This is the first description of the physicochemical properties of an androgen receptor in its purified form. (Funded by HD-12788, HD-00318, MDA Fellowship).

11. THE EFFECTS OF ST-1435 (ST) AND MIBOLERONE (MB) ON ANDROGEN BINDING PROTEIN (ABP) IN THE RAT. Russell R. Becker, Fred Moo Young, and C. Wayne Bardin. *Bowman Gray School of Medicine, Winston-Salem, North Carolina and The Population Council, New York, New York.*

Recent work suggests that medroxyprogesterone acetate (MPA) has a direct effect on the testis and that ABP levels may be used to measure such an influence on Sertoli cells. This study was undertaken to determine whether the synthetic norprogestin ST-1435 (16-methylene-17α-acetoxy-19-norprogesterone, Merck) would also have a direct effect on the testis as reflected by testicular, epididymal and serum ABP. Male rats received subcutaneous implants of ST and/or MB (17β-hydroxy-7α,17-dimethyl-estr-4-ene-3-one, Upjohn). Animals were killed at intervals; ABP was determined by RIA. ST produced a reversible decrease in testicular and epididymal weights. Although MB maintained the organ weights at control levels, its removal caused a drastic decrease. The hormones caused a suppression of LH, FSH, and testosterone. ST caused serum ABP to peak at 2–4 weeks; epididymal ABP content decreased below that of controls. For 4 weeks, testicular content was slightly elevated but suddenly fell below control levels. Thus, the elevation in serum ABP could have originated from either testis or epididymis. MB caused increased serum ABP, maintained epididymal ABP and elevated testicular ABP after 12 weeks. Upon MB withdrawal, ABP content of both organs fell. ST + MB produced an increase in serum ABP until treatment ceased. A drop in epididymal ABP was observed after 6 weeks of treatment. A peak in testicular ABP content was observed as with MB alone. These results suggest that ST, like MPA, has a direct effect on testicular function as reflected in serum and tissue ABP. The differential effects of ST and MB confirm that the mechanism which releases ABP into blood from Sertoli cells is different from that which transports ABP into the epididymis. (Supported by NIH grant 1P50 HD 13541).

12. EVIDENCE THAT MAJOR HISTOCOMPATIBILITY ANTIGENS ARE NOT EXPRESSED ON HUMAN SPERMATOZOA. Deborah J. Anderson and William C. DeWolf. *Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts.*

Products of the major histocompatibility complex constitute a family of polymorphic cell surface molecules that participate in important biological functions such as intercellular communication and immune regulation. Class I products (HLA-A,B,C in man) are classically thought to be expressed by all nucleated somatic cells; Class II products (HLA-D) have a limited distribution primarily on macrophages and certain lymphocyte subpopulations. Evidence for expression of MHC products in reproductive tissues (ie, the placenta and gametes) has been conflicting, but has remained a topic of intense interest because of the evolutionary significance of assigning the MHC a regulatory role in reproduction and

possible implications for genetic engineering of both Class I and Class II MHC products on human ejaculate sperm, but more recent studies have failed to detect these antigens on sperm and concern has been raised about the specificity of the antisera used in earlier studies, and the fact that seminal secretions contain HLA components that may bind to sperm. In this study we used a panel of highly specific monoclonal antibodies with well-defined reactivity against antigenic determinants on Class I and Class II HLA molecules to detect expression of MHC products on the surface of human ejaculate and epididymal sperm. In radioimmunoassay, Ficoll-purified ejaculate and epididymal sperm expressed less than 5% of the amount of HLA found on autologous lymphocytes. Autoradiography and fluorescence analysis of these samples revealed binding of monoclonal antibodies to contaminating mononuclear cells but not to sperm. Thus, we have ruled out the possibility that seminal plasma adds, masks or otherwise alters HLA molecules on sperm, and provide evidence that HLA-A,B,C and HLA-D antigens are not present on the surface of human sperm. (Supported by ACS IM-246 and NIH CA 28611 and 28775).

SESSION III

Clinical Andrology—A: Oral Presentations (13–18)

13. ANDROSTANEDIOL GLUCURONIDE (3αdiol G) IN PLASMA: A MEASURE OF PERIPHERAL ANDROGEN ACTION. R. Horton, R. Lobo, and D. Hawks. *Departments of Medicine and Gynecology, USC School of Medicine, Los Angeles, California.*

Circulating levels of testosterone (T) are generally in agreement with the clinical state. However, T is converted to DHT and to 3αdiol by sexual target tissues. In a recent report we indicated by direct kinetic analysis that DHT, 3αdiol G are generated outside of the liver, gut, splanchnic fat, and gonads (JCE 1981; 52:772). In the present study, we have examined a number of states in men and women and compared testosterone with those peripheral androgens. Total T was measured by RIA and "Free" T by SHBG precipitation or dialysis. 3αdiol and 3αdiol G in plasma were measured by specific RIA after chromatography. In a group of healthy and ambulatory elderly (>65) men, neither T nor 3αdiol values differed from those of younger men (<45 yrs). However, 3αdiol G falls with age (197 ± 68 to 96 ± 35 ng/dl, $P < 0.02$). Likewise, 3αdiol G in plasma is reduced in hypogonadal states.

Hirsutism in women was classified $1-4^+$ by Casey grading. Normal values are 35 ± 10 for T and <6.5 ng/dl for free T. 3αdiol is 4 ± 2 and 3αdiol G 40 ± 10 ng/dl. In those with PCO and $3-4^+$ hirsutism, all parameters were increased including 3αdiol G (867 ± 320 ng/dl). In idiopathic hirsutism, grading was $1-3^+$. Neither total nor free T correlated well with the clinical state (r = 0.1, T 52 ± 19 and free T 9.8 ± 5.7 ng/dl). 3αdiol was also not increased relative to the clinical state. However, plasma 3αdiol G was increased in IH and correlated with the clinical state (r = 0.8, 346 ± 104 ng/dl). Since we have also shown that plasma 3αdiol and 3αdiol G are derived from different pools, we conclude: plasma 3αdiol G is altered appropriately in clinical states of androgen reduction and excess. 3αdiol G may in certain disorders be superior to either total or free testosterone.

14. TESTICULAR ARTERIAL BLOOD SUPPLY IN VARICOCELE PATIENTS. F. Comhaire and M. Simons. *Department of Internal Medicine and Department of Nuclear Medicine, State University Hospital, B-9000-GHENT-Belgium.*

The physiopathological mechanisms underlying epididymal-testicular dysfunction in some patients suffering from varicocele disease are as yet unclear. We (Fertil Steril 1974; 25:88) have suggested reflux of renal/adrenal venous blood, containing a relatively high concentration of catecholamines, to be involved. Noradrenaline concentration was indeed found to be significantly higher in refluxing spermatic venous blood (mean 42 ng/ml) obtained during selective catheterization, than in peripheral venous blood (mean 17 ng/ml). Due to counter-current exchange, which may be enhanced by the increased hydrostatic pressure in the pampiniform plexus, the concentration of noradrenaline may be increased in the testicular arterioles, causing chronic constriction (Terquem, Int J Androl 1981). Testicular arterial perfusion was studied by means of ^{99m}Tc pertechnate injection. One third of 40 patients with varicocele and disturbed testicular function presented a reduced arterial blood supply at the left side (left/right ratio: 70–89%), rapidly normalizing after interruption of reflux by means of transcatheter embolization of the spermatic vein. In the remaining patients either symmetrical arterial perfusion (left/right ratio 90–120%) or very early reflux was found. It is concluded that testicular arterial vasoconstriction probably is not the sole cause of testicular dysfunction in varicocele disease.

15. HCG STIMULATION IN INFANTS WITH MICROPHALLUS AND HYPOPITUITARISM. Walter J. Meyer, III, Richard W. Furlanetto and M. Cassandra Matustik. *University of Texas Medical Branch, Galveston, Texas.*

The evaluation of microphallus in infants often includes a HCG stimulation test to access testicular function. After 5 days of HCG stimulation (3000 U/m², IM, qd), testosterone rises above 200 ng/dl in males with a normal hypothalamic-pituitary-testicular axis. In gonadotropin deficient patients, an inadequate response occurs after five days but a normal response occurs after six weeks of HCG stimulation (3000 U/m², IM, 3 × w). Three XY patients with microphallus (1.0, 1.8, 2.0 cm) and hypopituitarism were tested with a five day HCG stimulation test at two months of age and retested at

3–4 years of age (see Table). Two patients had a normal response at two months but a response typical of gonadotropin deficiency at 3–4 years of age. One patient had a borderline response on both occasions. After receiving six weeks of HCG at age 3, patient number 1's testosterone rose to 2083 ng/dl and his penis increased to 4.2 cm.

Testosterone (NG/DL) Pre and Post 5 Day HCG

Patient	2 Months		3–4 Years	
	Pre	Post	Pre	Post
1	20	478	8	95
2	34	356	6	6
3	10	206	11	239

These studies demonstrate that a five day HCG test is unreliable for determining gonadotropin deficiency in infants. We postulate that his unreliability is secondary to testicular priming with intrauterine maternal chorionic gonadotropin.

16. EFFECTS OF [D/LEU^6 DES-GLY-NH$_2^{10}$, PRO-ETHYLAMIDE^9]-GnRH (LEUPROLIDE) ON STEROIDOGENESIS WHEN USED TO TREAT PROSTATIC CARCINOMA. B. Warner, R. Santen, L. Demers. *Pennsylvania State University College of Medicine, Hershey, Pennsylvania.* D. Max, P. Lohmar. *Department of Clinical Research, Abbot Labs, N. Chicago, Illinois.* J. Smith, *University of Utah Medical Center, Salt Lake City, Utah.* H. Muss, M. Resnick, *Bowman Gray School of Medicine, Winston-Salem, North Carolina.* M. Glode, J. Wettlaufer, *University of Colorado Medical Center, Denver, Colorado.* M. Eisenberger, N. Block. *University of Miami, Miami, Florida;* and other investigators.

In animals, chronically administered superagonist analogues of GnRH decrease circulating androgens and gonadotropins; diminish androgen dependent tissue mass; and perturb steroidogenesis at several enzymatic steps (blocking 17α-hydroxylase and C-17-20 lyase; inducing 5α-reductase and 3-keto-reductase). Based on this pharmacology, a multicenter trial using the GnRH analogue leuprolide to treat prostatic carcinoma patients was initiated. Daily subcutaneous injections of leuprolide were administered to 87 patients (36 castrate and 51 intact), on whom gonadotropins, testosterone (T), and dihydrotestosterone (DHT) were measured. A subgroup of 12 intact patients had measurements of progesterone, 17α-hydroxy-progesterone, androstenedione (Δ^4), 3α-androstanediol (3α-diol), and estradiol. In response to drug administration, LH and FSH levels in castrate men increased acutely at 8 h but were suppressed by 2 wks and chronically. Similarly, LH levels in intact men rose from 21 ± 5 mIu/ml basally to 95 ± 21 at 8 h and then fell to 11 ± 2 at 2 wks. FSH concentrations were similarly affected (18 ± 5 mIu/ml basal, 46 ± 11 8 h, 8 ± 2 wks). Basal T (267 ± 39 ng/dl) also increased acutely (418 ± 100 ng/dl 72 h). The most striking finding, however, was the marked chronic inhibition of T, by 20-fold, into the castrate range chronically (11 ± 1 ng/dl 6 wks). Of further interest was the observation that DHT and 3α-diol were also suppressed, albeit to a lesser extent, a finding consistent with observations in rat testes. Both our rat and human studies strongly suggest thelegitimacy of leuprolide as a blocker of testicular androgen synthesis and as such support its use in androgen dependent states, particularly prostatic carcinoma.

17. POSSIBLE ROLE OF L-CARNITINE AND OF NEUTRAL α-GLUCOSIDASE IN THE DIAGNOSIS OF AN OBSTRUCTIVE PATHOLOGY OF THE REPRODUCTIVE TRACT IN INFERTILE MEN. Roland R. Tremblay, Pierre Chapdelaine, Réjean Roy, and Michel Thabet. *Departments of Medicine and Urology, Laval University, Quebec, Canada.*

The discovery of an epididymal pathology or of an obstructive process at the vas deferens level is of major importance in the treatment of subfertile males with severe defects of zoospermia. The goal of this preliminary study was to determine whether the measurement of specific activity of α-glucosidase and of L-Carnitine was helpful in prediction of a gross epididymal or vas deferens pathology. For this purpose both parameters were measured in the seminal plasma of severe oligo or azoospermic patients as well as in vasectomized men. Results reflect the range of values in each group.

	N	α-GLUCOSIDASE (μU/mg)	L-CARNITINE (mM)
Healthy subjects	80	700–2800	0.4–1.2
Vasectomized patients	60	0–700	0.1–0.4
Oligozoospermia	70	200–2800	0.1–0.7
Azoospermia	30	150–2800	0.1–0.5

The analysis of our data revealed that in both groups of oligo or azoospermic infertile men, 50% of α-glucosidase and/or L-Carnitine values were in the range of vasectomized patients. In ten of these cases with such abnormalities, a diagnosis of bilateral agenesis or obstruction of the vas deferens was firmly established by vasography and/or bilateral scrotal exploration. In some cases of oligozoospermia, L-Carnitine was more significantly decreased than α-glucosidase, this latter component of the epididymal fluid remaining in correlation with zoospermia. It is therefore proposed that when supportive biochemical evidences are available, vasography and/or bilateral scrotal exploration should be performed in an ultimate effort to identify and possibly cure an obstructive disease of epididymis or vas deferens. (Supported by grant MT-4780 of Medical Research Council of Canada).

18. THE INCIDENCE OF SPERM-ASSOCIATED IgG AND IgA IN A LARGE INFERTILE MALE POPULATION. G. G. Haas Jr., R. Weiss-Wik, *Pennsylvania School of Medicine, Philadelphia, Pennsylvania.*

We have previously reported a direct radiolabeled antiglobulin test to quantitate specific antibodies on sperm. Previously, patients were tested for sperm-associated antibodies because of the presence of plasma IgG antisperm antibodies or the infertility work-up suggested antisperm antibodies (ie abnormal postcoital test, poor semen analysis, etc). The incidence of sperm-associated antibodies in the entire infertile male population cannot be derived from this data if plasma antibodies do not reliably correlate with similar antibodies on the sperm surface. In this report the percentage of infertile males with increased sperm-associated IgG and IgA was quantitated in all men undergoing a routine semen analysis during their infertility evaluation. In our initial result 2 (9.5%) of 21 males had markedly increased sperm-associated IgG (greater than 3× fertile controls); one (4.8%) of these men also had an increased sperm-associated IgA. Seven (33%) men were found to have 2× but less than 3× sperm-associated IgG found on fertile controls; one (4.8%) of these men also had a marginally increased sperm-associated IgA. There did not appear to be an association between semen analysis parameters and the results of sperm-associated antibody testing in these early patients tested. Plasma from men positive for sperm-associated IgG and IgA were assayed for plasma antisperm antibodies and the percentage of false negative results if only plasma antibodies had been assayed was calcualted.

SESSION IV

Hormonal Regulation and Drug Effects: Oral Presentations (19–24)

19. ANDROGEN REGULATION OF PROSTATIC POLYAMINE CONTENT IN AGING AXC RATS. Sydney A. Shain and Chris M. Lancaster. *Southwest Foundation for Research and Education, San Antonio, Texas.*

We examined the effects of aging and orchiectomy or testosterone treatment on prostatic polyamine content. We did not observe a consistent effect of aging. Putrescine and cadaverine content of ventral prostate of aged (25.7-month-old) AXC rats was diminished 34 and 28 percent respectively when compared to mature (5.3-month-old) AXC rats. Dorsolateral prostate putrescine and cadaverine content respectively was 20 and 11% of that of ventral prostate and was age invariant. Spermidine and spermine content of ventral prostate of aged AXC rats was increased 28 and 40%, respectively, whereas dorsolateral prostate spermidine and spermine content was decreased 37 and 32%, respectively, when compared to mature AXC rats. The effectiveness of orchiectomy as a means of decreasing prostatic polyamine content was significantly diminished in aging AXC rats. Treatment of mature AXC rats with testosterone propionate (2.5 mg sc at 48 and 24 hours prior to sacrifice) elevated ventral prostate putrescine and cadaverine content, had no effect on spermidine content, and diminished spermine content. Dorsolateral prostate putrescine and cadaverine content was unchanged and spermidine and spermine content was diminished in testosterone treated mature AXC rats. Testosterone treatment had no effect on polyamine content of ventral or dorsolateral prostates of aged AXC rats. Comparison of these data with data for prostatic L-ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (AMDC) activity of identically treated AXC rats of comparable ages showed there was no linkage between prostatic polyamine content and activity of the enzymes, ODC and AMDC, of polyamine biosynthesis. Our studies suggest that polyamine content of differentiated, nonproliferating prostatic cells is not stringently controlled by the activity of ODC and AMDC and that the bulk of prostatic polyamines is not intimately associated with regulation of differentiated prostate cell function.

20. REGULATION OF RAT TESTICULAR ORNITHINE DECARBOXYLASE (ODC) ACTIVITY *IN VITRO*: EFFECT OF AGE, FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH). J. Osterman, E. Murono, T. Lin, and H. R. Nankin. *Medical Service, Wm. Jennings Bryan Dorn Veterans' Hospital and Department of Medicine, University of South Carolina School of Medicine, Columbia, South Carolina.*

The role of polyamines as possible regulators of testicular function is poorly understood. Previous *in vivo* studies in immature rats showed that both FSH and LH stimulate the activity of testicular ODC, the rate-limiting enzyme of polyamine synthesis. The present studies explore hormonal regulation of ODC activity of decapsulated rat testis, testicular interstitial cells and purified Leydig cells under defined conditions *in vitro*. Both immature (15–26 day old) and adult (60–90 day old) rat testes were employed. Basal (fresh tissue) ODC activity varied widely among rats of the same age but was similar (<10% difference) in pairs of testicles from the same animal. For this reason pairs of testes were compared in subsequent *in vitro* studies. ODC activity of decapsulated testes of immature rats remained stable (vs fresh tissue) during 4 h incubation in Medium 199 + 0.1% bovine serum albumin + 0.1 mM 1-methyl,3-isobutyl-xanthine at 34°. FSH (100 ng/ml) caused a small but statistically significant ($P < 0.005$) stimulation of the enzymatic activity and 8-bromo cAMP (0.5 mM) mimicked the effect of FSH. In contrast, ODC activity of decapsulated testes of adult rats sharply declined (to 25–30% of basal) during 4 h of incubation. Addition of FSH, LH, prolactin, prostaglandin E_2 (PGE_2), epidermal

growth factor (EGF), 10% fetal calf serum or insulin singly or in combination failed to prevent ODC activity decline. In isolated interstitial cells and purified Leydig cells from adult rats LH stimulated ODC activity in a dose (10–200 ng/ml) and time-dependent fashion. 8-Bromo-cAMP mimicked the effect of LH. Prolactin, insulin, PGE_2 or EGF were without effect. This study demonstrates for the first time direct *in vitro* stimulation of rodent testicular ODC activity by gonadotropins and reveals marked age-dependent differences in *in vitro* regulation of this enzyme.

21. DURATION OF STERILITY IN MICE AFTER TREATMENT WITH RADIATION OR CYTOTOXIC DRUGS. Marvin L. Meistrich and Marcia V. Finch. *University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.*

The duration of sterility of male mice was measured after treatment with irradiation or with chemotherapeutic drugs. It was shown to be correlated with spermatogenic stem cell survival and testicular sperm production. Stem cell survival was measured by counts or repopulating seminiferous tubular cross sections 35 days after treatment. Treatment with those drugs which did not produce sufficient stem cell killing to be detected by this method, resulted in sterile periods ending less than 85 days later. Gamma radiation, Adriamycin®, and thiotepa produced both extensive stem cell killing and longer periods of sterility. The duration of sterility was highly correlated ($r = 0.91$) with stem cell survival for all three agents. Thus, by using a short term (35 days) assay for stem cell survival, we can predict the duration (85–218 days) of sterility. The same relationship between the duration of sterility and stem cell survival holds for all agents tested. Therefore, we suggest that stem cell killing is solely responsible for the observed sterility and that possible damage to the endocrine systems or somatic cells of the testis, does not contribute significantly to the sterility. In addition, testicular sperm production was measured at various times during recovery of spermatogenesis. At the time of return of fertility, sperm production was, in all cases, about 10% of control levels. This observation further indicates that possible effects of the treatment on sperm transport, accessory glands, libido, and sperm quality are not as important as the level of sperm production in determining the fertility of these mice. (Supported by grants CA-17364 and CA-06294 from the National Cancer Institute).

22. THE EFFECT OF TESTOSTERONE ON THE INDUCTION OF TWO MESSENGER RNAs CODING FOR TWO ABUNDANT PROTEINS IN THE RAT SEMINAL VESICLE SECRETION. Per-Erik Mansson, Donald Carter, and Stephen Harris. *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.*

A small set of abundant testosterone regulated poly peptides in the seminal vesicle secretion (SVS IV, V and IV) have been used as markers for androgen action by several investigators. We have studied the induction by testosterone of the mRNAs coding for protein IV and protein V. Rats castrated for five weeks were injected daily with 1 mg testosterone propionate. At different time intervals, rats were killed and the seminal vesicles were removed. Total cellular RNA was then isolated. Complementary DNA (cDNA) was synthesized to the abundant class of poly(A^+)RNA and cloned into PBR322. Two cDNA inserts that arrested the translation of mRNA IV and mRNA V were used as probes to determine the amount of the two mRNAs in total RNA. The level of mRNA IV and mRNA V in seminal vesicles from castrated rats was found to be between 25–250 molecules/cell. Four hours of testosterone increased the level significantly and 24 hours of hormone treatment resulted in 7,000 to 16,000 mRNA IV molecules and approximately 5,000 mRNA V molecules. After 96 hours of hormone treatment, the level of mRNA IV and mRNA V was approximately 50% and 30%, respectively, of the level found in seminal vesicles from normal rats, which indicate that the rate of induction of mRNA V is roughly 2-fold slower than that of mRNA IV. The level of induction of mRNA IV and mRNA V in rat seminal vesicle was found to be similar to the induction of abundant mRNA in rat ventral prostate [Parker MG et al. J Biol Chem 1980; 255:6996–7001] and the induction of ovalbumin in chick oviducts [Harris, SE et al. Biochemistry 1975; 14:2072–2081]. Presently we are sequencing the cDNA V insert in order to study the absence/presence of homology with the cDNA IV sequence.

23. EVIDENCE FOR PROGESTERONE RECEPTORS IN THE EPIDIDYMIS OF THE PREPUBERTAL DOG. Lovell A. Jones and Carolyn J. Connell. *University of Texas, Systems Cancer Center, Houston, Texas, and Colorado State University, Fort Collins, Colorado.*

We have demonstrated high affinity binding sites for progesterone in the epididymides of prepubertal laboratory Beagles. It has been well documented that estrogen treatment significantly stimulates the appearance of progesterone receptors (Dube et al, 1979). We have examined epididymides of pups injected with estrogen (100 μg of 1, 3, 5, (10)-estratrien-3,17βdiol 3-benzoate in sterile sesame oil daily from ages 2 to 20 weeks), sterile saline or sesame oil, LH (Papkoff, dog G-3-222b, 1.8 μg per day) and FSH (Papkoff, dog G-4 150 C, 1.8 μg per day). These injections were given into the vaginal cavity of the scrotal sac in 0.18 ml of vehicle, alternating between left and right sides. Epididymides were removed at surgery and immediately frozen and stored in liquid nitrogen until assayed. Estrogen and progesterone receptors were assayed using the hydroxyapatite method (Hoffman et al, 1980) with ^{3}H estradiol and ^{3}H progesterone lignads. Means ± SE binding for each receptor (fmol/mg. of protein) were as follows:

Treatment:	SALINE		OIL		E_2B		FSH		LH	
Age (wks.)	16	20	16	20	16	20	16	20	16	20
E_2B Receptors	119	132	—	164	—	65	—	132	193	88 (b)
Prog Receptors	16	49	—	49	—	185	—	103	30	72

The binding of progesterone in the epididymis is of high affinity (k_d of 1×10^{-9} to 10^{-10}M). Treatment with E_2B and FSH elevated the total progesterone receptor binding in the epididymis while E_2B and LH_b lowered the estradiol binding. CHronic estradiol treatment has a profound effect on the development of the canine epididymis (Connell and Donjacour, 1981) that may be mediated by wat of a receptor mechanism. The presence of progesterone receptors in the epididymis that are E_2B sensitive suggests that these receptors have a role in the development of the structure and function of the canine epididymis. (Funded in part by NIH Grant HD13928 and BRSG 2 S07 RR-05458-19).

24. CULTURE OF IMMATURE RAT PROSTATE EPITHELIAL CELLS. Albert F. Clark, John Orlowski, and Charles E. Bird. *Departments of Biochemistry and Medicine, Queen's University, Kingston, Ontario, Canada.*

To initiate studies on the androgenic control of rat prostatic acid phosphatase (AP) production, experiments were begun to establish a suitable short term culture system of immature rat prostate cells. The immature rat prostates are digested with a collagenase-trypsin mixture. Epithelial cells are separated from stromal cells using Percoll gradients. Eagle's minimum essential medium containing fetal bovine serum (FBS) and testosterone supported only minimal growth of the epithelial cells with there being no cell attachment. However culture of the cells in F-12/DME medium containing 15% FBS plus testosterone, insulin, dexamethasone, retinoic acid and transferrin led to the appearance of a number of colonies attached to the culture dish. When testosterone was absent in the culture medium there were only a few small colonies. The colonies in medium containing androgen stained intensely for Ap activity when compared to the small colonies grown in the absence of testosterone. As well, the ability of the cells to reduce testosterone to 5α-dihydrotestosterone was greater for the cells grown in the presence of testosterone. This culture system thus has two *in vivo* characteristics for the study of androgen mediated actions—AP and Δ^4-5α-reductase activities. (Supported by MRC and NCI of Canada).

SESSION V

Fertility Regulation: Oral Presentation (25–30)

25. THE RELATIONSHIP OF PROTEIN CARBOXYL-METHYLASE TO MOTILITY AND FERTILITY OF HUMAN SPERMATOZOA. B. Jane Rogers and Jane Nelson. *University of Hawaii School of Medicine, Honolulu, Hawaii.*

Protein carboxyl-methylase (PCM), an enzyme that catalyzes the transfer of activated methyl groups from S-adenosyl methionine to ionized carboxyl groups on proteins, has been localized in the sperm tail and may be an important component of the motility apparatus. PCM activity was reported to the reduced in the sperm of necrospermic patients as compared to normal patients (Gagnon et al, 1980). Because of its potential usefulness as a biochemical index for both motility and fertility, PCM levels were measured in patients and donors with motilities ranging across the whole spectrum (0–80%). Fertility was measured by the zona-free hamster egg penetration assay. PCM activity was assayed in cytosol preparations derived from triton-treated sperm. PCM activity is expressed as picomoles of tritiated S-adenosyl methionine/mg protein. Our results confirm that PCM activity is related to motility but only when the extreme ends of the spectrum are considered. Patients with non-motile sperm had low PCM values (2.2–6.1) whereas normal donors with motility greater than 50% had high PCM values (43–105). A similar relationship did not exist however between PCM and fertility. Individuals with high PCM had fertilization rates ranging from 0 to 100%. Correlations were not demonstrable between PCM and motility or fertility at the intermediate levels of motility (20–60%). This lack of correlation of PCM activity with either motility or fertility underscores the inadequacy of motility determinations is diagnosing fertility. (Supported by NIH grant HD 11555).

26. IS THE OUTCOME OF PREGNANCY IN INFERTILE COUPLES RELATED TO SEMEN QUALITY? Luis J. Rodriguez-Rigau, Keith D. Smith, and Emil Steinberger. *Department of Reproductive Medicine and Biology, University of Texas Medical School, Houston, Texas.*

It has been suggested in the past that poor semen quality, particularly increased percent of morphologically abnormal spermatozoa, may be responsible for early spontaneous abortion. To investigate this possibility we analized the correlation of semen quality to the outcome of 436 consecutive pregnancies in our infertility practice. Early spontaneous abortion occurred in 86 cases (19.7%), 348 women carried the pregnancy to term, and there were two tubal pregnancies. The incidence of spontaneous abortion in 131 pregnancies resulting from AID (artificial insemination with donor semen) was 21.4%, not significantly different from the 19.0% spontaneous abortion rate in the 305 non-AID pregnancies. The average sperm count (S.C.) motile sperm count (M.S.C.), total sperm count (T.S.C.), total motile sperm count (T.M.S.C.), percent abnormal spermatozoa and incidence of varicocele were not significantly different

in cases where the pregnancies ended in spontaneous abortion and those that were carried to term.

	Term pregnancy	Spontaneous Abortion
S.C. (10^6/ml)	71.7 ± 4.6	80.0 ± 10.9
M.S.C. (10^6/ml)	51.3 ± 3.9	62.8 ± 9.8
T.S.C. (10^6/ejaculate)	196.0 ± 13.8	242.4 ± 33.0
T.M.S.C. (10^6/ejaculate)	142.1 ± 11.6	195.0 ± 29.4
% Normal sperm	59.1 ± 3.6	60.8 ± 4.1
% Varicocele	26.8	22.6

The results of this study do not suggest that abnormal semen quality is associated with increased incidence early spontaneous abortion in infertile couples. (This study was supported by grant #6-168 from the National Foundation-March of Dimes).

27. SPERMATOGENIC FUNCTION IN THE VASECTOMIZED MONKEY (*Macaca fascicularis*).

Mark A. Hadley and Martin Dym. *Department of Anatomy, Georgetown University, Washington, D.C.*

Earlier observations of the seminiferous epithelium in the vasectomized monkey have indicated that spermatogenesis is generally unimpaired. There are, however, reports of focal degenerative changes; these include the presence of occasional seminiferous tubules containing only Sertoli cells and spermatogonia, duplication and infolding of the basal lamina and increased Sertoli cell phagocytosis of spermatids (Chapman, et al, Anat Rec 1978; 192:41). More recently, Tung and Alexander (Am J Pathol 1980; 101:17) reported a greater prevalence of testicular disease, resembling allergic orchitis, in rhesus monkeys vasectomized as long as 15 years. Their age matched controls, however, also displayed similar lesions but to a lesser extent. The objective of our study was to determine quantitatively whether there is any alteration in the germinal epithelium in the adult vasectomized cynomolgus monkey which may lead to impaired spermatogenic function. Quantitative analysis of the germinal epithelium (stage VII of the spermatogenic cycle) and seminiferous tubule diameter measurements were performed on bilaterally vasectomized and bilaterally sham operated monkeys at postoperative intervals of ten and 18 months. The morphology of the testes from vasectomized monkeys appeared normal compared with controls, lacking the apparent pathological changes reported in earlier works. The total number of spermatogonia (Ad and Ap), preleptotene spermatocytes, pachytene spermatocytes, and spermatids per seminiferous tubule cross section did not vary significantly between the control and vasectomized groups. The number of each germ cell type per Sertoli cell nucleoli and the mean seminiferous tubule diameter were also found not to vary significantly between the control and the vasectomized groups. Our study demonstrates quantitatively that spermatogenic function in the cynomolgus monkey is not inhibited up to 18 months following vasectomy. (Supported in part by NIH grant HD-11606).

28. ANTIGENIC PEPTIDE FRAGMENTS OF THE SPERM SPECIFIC LDH-C_4.

Thomas E. Wheat, Victoria Gonzales-Prevatt and Erwin Goldberg. *Department of Biological Sciences, Northwestern University, Evanston, Illinois.*

Lactate dehydrogenase C_4 (LDH-C_4, LDH-X) is a spermspecific isozyme which is an effective antigen in the immunosuppression of fertility. Immunization of female mice, rabbits and baboons with the enzyme purified from mouse testes results in a significant reduction in pregnancies. This effect is titer related and reversible. Apparently, humoral antibodies to LDH-C_4 appear in reproductive tract fluids as a serum transudate and interact with the antigen on the sperm surface. This causes agglutination of spermatozoa and effectively blocks fertilization. In order to replace the natural product in the development of a contraceptive vaccine, peptides bearing antigenic determinants of LDH-C_4 are being tested as synthetic immunogens. We have previously described the isolation of one such peptide, MC152-159. A synthetic analogue of this peptide conjugated to BSA elicited an immune response which included antibodies to the native protein. We have now isolated 10 additional peptides which are three to five times more active than MC152-159 in binding anti-LCH-C_4. The amino acid sequences of these peptides were partially or completely determined by the Edman degradation. Synthetic preparations of MC_{5-15} and MC_{44-58} have been conjugated to BSA and injected into rabbits in order to provoke an immune response to native LDH-C_4. Peptides MC_{61-77}, MC180-210, and $MC_{283-317}$ are being subjected to additional cleavage procedures to more precisely delineate the residues responsible for antibody-binding. The remaining immunologically active peptides cannot yet be placed in the overall structure of LDH-C_4. The studies demonstrate the feasibility of replacing native LDH-C_4 with a synthetic peptide fragment in the development of a contraceptive vaccine. (Supported by grants from NIH and a contract with PARFR).

29. GOSSYPOL ACETIC ACID INHIBITION OF ISOLATED BOVINE SPERM PLASMA MEMBRANE Ca^{2+}-ATPASE.

K. J. Whaley and D. W. Bishop. *Department of Physiology, Medical College of Ohio, Toledo, Ohio.*

Many cell processes are modulated by intracellular calcium concentration, which is regulated by membrane-bound Ca^{2+}-ATPase activity. Interest in the precise mechanism of gossypol action and the demonstrated effects of this antifertility agent on total sperm Ca^{2+}-ATPase (Kalla and Vasudev, 1981) warranted further investigation of the mechanism and localization of the inhibition. Hypotonically treated bovine sperm from ejaculates, caput and cauda epididymides yield plasma membranes with significant Ca^{2+}-ATPase activity. This ouabain-insensitive enzyme is inhibited 80% by gossypol acetic acid (10 μM). Gossypol inhibits Ca^{2+}-

ATPase in membranes prepared from sperm from all three sites of the reproductive tract. The enzyme is also inhibited 50% by cholesterol (0.4 mg/ml incubation medium) and 40% by 5p μM trifluoperazine, an inhibitor of calmodulin. The plasma membrane marker, 5'-nucleotidase, is not inhibited by gossypol (10 μM), cholesterol (0.4 mg/ml) or trifluoperazine (50 μM). Gossypol inhibition of plasma membrane-bound Ca^{2+}-ATPase indicates that part of the mechanism responsible for infertility may be direct action of gossypol on sperm plasma membrane function.

30. THE MECHANISM OF ORDRAM®'S ANTIFERTILITY EFFECT IN MALE RATS. J. M. Killinger, J. L. Minor, P. D. Royal, R. A. Williams, J. R. Downs, and G. M. Zwicker. *Environmental Health Center, Stauffer Chemical Company, Farmington, Connecticut.*

Ordram, a rice herbicide, produces an antifertility effect in male rats. Its site of action was investigated. In part I, male rats received either 0, 12, or 60 mg/kg of Ordram for five days, then they were mated with one female per week for ten weeks. The results of this study phase identified late spermatid development as the stage of spermatogenesis sensitive to Ordram exposure. This was concluded from the substantial reduction in male fertility during the third post-treatment week. The no-effect level was 12 mg/kg. In part II, male rats were treated with either 0, 0.2, 4, 12, or 30 mg/kg of Ordram for five weeks, then they were mated with two females per week for one week. At terminal sacrifice, blood, sperm samples from the cauda epididymis, and reproductive tissues were taken for evaluation. A comparison was made between dose, fertility, changes in plasma hormone levels (testosterone, FSH, LH, TSH, T_3, and T_4), sperm morphology, motility and viability, and morphologic changes in the testes and epididymides. There were no measurable treatment-related changes in serum hormone concentrations which correlated with the reduction in male fertility. The dose-response relationship observed for male fertility could be correlated with changes in sperm viability, morphology, motility, and concentration. Electron micrographs indicate that the site of action may involve the sperm plasma membrane. Histological examination of the testes and epididymides revealed a slight increase in degenerating spermatids in small portions of a few tubules. A clear dose-response relationship was observed at 4, 12, and 30 mg/kg with 0.2 mg/kg being the no-effect dose level in this study. In part III, Ordram's effects on sperm from the caput and cauda epididymis were compared. (Protein RIA kits were obtained from NIAMDD, DR. A. F. Parlow).

SESSION VI

Physiology, Developmental and Age-Related Studies: Oral Presentations (31–36)

31. EFFECT OF CRYPTOCHIDISM AND ORCHIDOPEXY ON INHIBIN SECRETION BY RAT SERTOLI CELLS. L. Seethalakshmi and Anna Steinberger. *Department of Reproductive Medicine and Biology, University of Texas Medical School, Houston, Texas.*

Cryptorchidism leads to impaired spermatogenesis, Sertoli cell function and elevated gonadotropin levels. Steinberger (1980) reported suppression of inhibin secretion by isolated Sertoli cells culcutred *in vitro* after 25d of bilateral cryptorchidy. The present study was undertaken to further explore the effect of cryptorchidy and orchidopexy on the inhibin secretion. Matire, bilaterally cryptorchid rats were sacrificed at 21d, 28d, and 35d after surgery. In some 35d cryptorchid animals, the testes were returned to the scrotum for 14d or 42d before sacrifice. Blood was collected for FSH and LH radioimmunoassay. The testes were weighed and portions fixed for histology. The Sertoli cells were isolated and cultured at 32 C in medium containing 10% fetal bovine serum. Sertoli cell protein and DNA were determined to monitor culture uniformity. Spent media from 3d and 6d cultures were bioassayed for inhibin activity using rat pituitary cells. Progressive loss of germ cells associated with increasing serum levels of FSH and LH, occurred with time of cryptorchidy. By 42d after orchidopexy, spermatozoa reappeared and serum FSH returned to near normal level, while LH remained 2× above normal, suggesting differences in their feedback regulation. The results of inhibin bioassay revealed a progressive decrease of inhibin secretion to nondetectable level by 35d of cryptorchidy. The inhibin secretion returned to control values after 42d of scrotal recovery. These results clearly indicate that bilateral cryptorchidism reduces the ability of Sertoli cells to secrete inhibin, which could account at least partly for the increases in circulating FSH. Impaired Sertoli cell function may also contribute to the germ cell losses. However, all of the effects of cryptorchidism were reversed by 42d after orchidopexy. (Supported by NIH Center Grant P50 HD 08338).

32. EPISODIC LH SECRETION IN PREPUBERTAL AND POST PUBERTAL CASTRATE MALE RATS. A. E. Karpas, A. M. Matsumoto, R. A. Steiner, D. K. Clifton, and W. J. Bremner. *Departments of Medicine, Obstetrics-Gyncology and Physiol-Biophysics, University of Washington School of Medicine and VA Medical Center, Seattle, Washington.*

Current concepts of puberty in mammals include an important role for maturation of the central nervous system (CNS) control mechanisms for pulsatile LH secretion. To determine the functional characteristics of the CNS control mechanisms independent of gonadol steroid feedback, we have determined the frequency

and amplitude of episodic LH secretion in prepubertal and postpubertal castrate male rats. Male Sprague-Dawley rats were divided into two groups: prepubertal (age 49 days, n = 14) and adult (age 70–90 days, n = 24). Seven prepubertal animals and all the adults had been castrated 21–25 days prior to study. The other seven prepubertal rats were left intact. In the castrate prepubertal and adult rats, blood sampling was performed at 4 min intervals for 4 hr from indwelling jugular cannulae while the animals were awake and unrestrained. Whole blood LH was measured in duplicate by RIA and episodic LH secretion was analyzed by the cycle detection computer program (Clifton and Steiner, *Endoc Soc Abst* 940, 1981). The results are shown in the table.

	Mean LH	Interpulse Interval	Pulse Amplitude
Prepubertal	372 ± 38 ng/ml	16.4 ± 1.5 min	109 ± 1 ng/ml
Adult	350 ± 13	19.1 ± 0.9	117 ± 8
	N.S.	N.S.	N.S.

Serum testosterone levels (0.84 ± 0.13 ng/ml) measured by RIA on the intact animals at 49 days of age confirmed that rats of this age were prepubertal. Conclusions: Mean LH levels, LH pulse frequency, and amplitude did not differ significantly between late prepubertal and adult castrate rats, implying that, in the absence of gonadal feedback, CNS-pituitary control mechanisms function similarly in these two age groups. An important remaining question is the effect of physiological levels of gonadal steroids on pulsatile LH secretion before and after puberty.

33. TESTICULAR RECRUDESCENCE IN THE GOLDEN HAMSTER AS A POSSIBLE MODEL OF PUBERTY.

Albert S. Berkowitz and Jerrold J. Heindel. *Department of Reproductive Medicine and Biology, The University of Texas Medical School at Houston, Houston, Texas.*

The goal of this study was to determine whether testicular recrudescence in the golden hamster can be utilized as a model of puberty. We have therefore measured the ability of FSH to stimulate cAMP accumulation in Sertoli cells cultured *in vitro* from hamsters during sexual maturation and testicular recrudescence, and correlated it with changes in serum gonadotropins. The maximal response of Sertoli cells to FSH from 18, 30 and 60 day-old hamsters declined from 2241 ± 245 to 684 ± 64 and 418 ± 61 pmol cAMP/mg p, respectively. Serum FSH levels changes during sexual maturation rising to a peak at 30 days of age (840 ± 200), and then declining to adult values. LH serum values parallel FSH levels with a lag time of approximately five days. In order to examine these same parameters during testicular recrudescence, hamsters were blinded to induce testicular regression. During regression testicular weight declined from 1980 to 270 mg/testis with a concomitant decline in gonadotropins. The FSH responsiveness of Sertoli cells correlated inversely with the weight of the testes such that cells from fully regressed testes responded to FSH with a maximal response of 2908 ± 78 pmol cAMP/mg p. During recrudescence the testes weight spontaneously increased, Sertoli cell response to FSH *in vitro* declined and the serum FSH level increased to a peak of over 1300 ng/ml and then returned to the adult level. Serum LH levels also paralleled the pubertal pattern. On the basis of this correlation between testicular weight, gonadotropin levels, and Sertoli cell cAMP response to FSH *in vitro*, we propose that puberty and testicular recrudescence are physiologically similar events and that testicular recrudescence in the hamster may be a useful model of puberty. (Supported by NICHD grant 5P50-HD08338, BRSG grant SO7 RR05745-06 and NSF PCM 78-24541).

34. EXPERIMENTAL UNILATERAL CRYPTORCHIDISM: IS THERE A CONTRALATERAL EFFECT ON THE DESCENDED TESTIS?

Stanley Kogan, Fikret Vatandaslar, Bernard Gondos, Boyce Bennett, Sandra Nehlsen, Eric Sun, Adrienne Fleckman, Paul Smey and Selwyn Levitt. *Albert Einstein College of Medicine, Bronx, New York and University of Connecticut Health Center, Farmington, Connecticut.*

Both clinical and experimental data suggest that unilateral testicular disease may adversely affect the contralateral testis. In this study, Sprague-Dawley rats were made unilaterally cryptorchid (UC) or monorchic (M) within 48 hours of birth to evaluate the effects of unilateral testicular disease on the contralateral normally descended testis (DT). At 33 and 66 days, plasma gonadotropins and circulating antibodies were measured, as well as testicular weight (TW), light, electron microscopy and immunofluorescence of the DT's. Weight of the DT (mg testis/gm body wt × 10^3) were 5.38 ± 0.23 in controls and 5.66 ± 0.44 in UC, compared with 7.18 ± 0.55 in M and 1.73 ± 0.75 in bilaterally cryptorchid (BC) rats. Thus, UC did not result in compensatory hypertrophy or atrophy. Despite absence of hypertrophy, at 66 days plasma LH was significantly higher than control rats (LH = 0.13 ± 0.04), in M (0.3 ± 0.12) and UC (0.23 ± 0.08), but lower than that seen in BC. FSH levels were elevated at 33 days in M, but not UC, and at 66 days were only elevated in BC. DT's from UC were normal by light and electron microscopic examination. Serum assayed for anti-rat complement dependent lymphocytotoxic antibodies and red cell agglutinins gave similar results for C, M and UC animals. Immunofluorescent staining of the DT and UDT failed to show deposits of C^3, IgM and IgG. These studies have demonstrated increased LH levels in M and UC rats. No induced morphometric, histologic or immunologic abnormalities were detected in the DT.

35. TESTICULAR PRL AND hCG BINDING, AND SERUM GH IN MALE GOLDEN HAMSTERS: EFFECTS OF PHOTOPERIOD AND TIME OF DAY.

H. Klemcke,

A. Bartke, and K. Borer. *University of Texas Health Science Center, San Antonio, Texas, and University of Michigan, Ann Arbor, Michigan.*

Injection—during daylight but not darkness—of PRL into hamsters kept in a short photoperiod (PP), caused a significant increase in testicular weight and LH binding (Endocrinology 1978; 103:2069). Injection of GH into these gonadally regressed hamsters increased testicular LH binding (Endocrinology 1978; 103:2069), but plasma GH was unaffected by PP (Endocrinology 1980; 106:167). In an attempt to ascertain if circadian changes in testicular PRL binding or serum GH might explain such data, the following study was conducted. Mature hamsters were kept on 14:10 (light:dark) or 5:19 PPs for 12 weeks. Hamsters on both PPs were sacrificed at 6 hr intervals. At time intervals measured, no significant changes ($P > 0.05$) in any parameters occurred during a 24 hr period. Also, neither serum GH, nor PRL binding sites were affected by PP. Concentration of hCG binding sites was greater ($P < 0.05$) in 5:19 vs 14:10 hamsters. Results (mean ± SEM) are presented below:

Parameter Measured	Photo-period	Time of Day 0400	1000	1600	2200
oPRL Binding	14:10	13 ± .9	11 ± .9	12 ± 1	13 ± 1
(fmol/mg protein)	5:19	11 ± 2	13 ± 2	15 ± 2	14 ± 2
Serum GH	14:10	4 ± .6	4 ± .2	16 ± 11	5 ± 2
(ng/ml)	5:19	29 ± 16	14 ± 8	7 ± 2	8 ± 3
hCG Binding	14:10	3 ± .2	3 ± .4	3 ± .4	3 ± .2
(fmol/mg protein)	5:19	10 ± 1	10 ± 3	13 ± 2	9 ± 3

These data indicate the probability that changes in PP do not affect serum GH concentrations. Short PP had no apparent effect on PRL binding site concentration when measured in whole testes tissue preparations, and circadian fluctuations in testicular sensitivity to exogenous PRL cannot be explained by putative changes in receptor concentration.

36. TESTOSTERONE AND PATTERNS OF LH SECRETION IN MALE SHEEP. M. J. D'Occhio, B. D. Schanbacher, and J. E. Kinder. *Department of Animal Science, University of Nebraska, Lincoln, Nebraska, and RLHUSMARC, SEA-AR, U.S. Department of Agriculture, Clay Center, Nebraska.*

The acute castrate ram (wether) was used in an experimental model to investigate the finer control of LH secretion by testosterone (T). At the time of castration mature animals received subdermal Silastic implants (3.35mm ID × 4.65mm OD) filled with crystalline T and either 15cm (0.5T implant) or 30cm (T implant) in length. Treatments were as follows (n = 3): no implants (rams, control wethers); one 0.5T implant, two T implants, four T implants. LH and T secretory patterns together with LH responses to LHRH (5ng/kg) were characterized six weeks later by bleeding all animals at 10 min intervals for 24 hr. Mean T levels (ng/ml, mean ± SEM) were: rams (2.25 + 0.49); wethers ($0.22 \pm 0.01^{a,*}$); 0.5T ($0.54 \pm 0.01^{a,*}$); 2T ($1.11 \pm 0.08^{c,*}$); 4T (2.01 ± 0.09^{d}) ($^{a,b,c,d}P < 0.05$, ANOVA;$^{*}P < 0.05$, different from rams). Mean LH (ng/ml) and number of LH pulses per 24 hr were, respectively; rams (2.1 ± 0.2^{a}; 3.3 ± 1.2^{a}); wethers (13.3 ± 5.2^{b}; 29.5 ± 0.5^{b}); 0.5T (21.3 ± 4.1^{b}; 27.7 ± 1.8^{b}); 2T (13.3 ± 1.0^{b}; 20.3 ± 0.09^{c}); 4T (2.2 ± 0.8^{a}; 2.7 ± 1.8^{a}). Peak LH responses (ng/ml) to LHRH were: rams (10.8 ± 0.9^{a}); wethers (36.3 ± 5.8^{b}); 0.5T (51.7 ± 4.0^{c}); 2T ($23.4 \pm 7.3^{a,b}$); 4T (13.1 ± 2.3^{a}). LH pulses occurred episodically in rams and at frequent rhythmic intervals in wethers. At a low dose T appeared to have a positive feedback action on LH. Serum T levels intermediate between those of wethers and rams caused a decrease in rhythmic LH pulses without an effect on mean LH. Physiological levels of T caused a dramatic decline in the incidence of LH pulses as well as mean LH levels. These results indicate that T feedback on LH in male sheep is not an all-or-none effect. Rather, dose-response relationships exists between serum T concentration and the pattern of LH secretion. The results also show that T feedback exists at both the hypothalamic and pituitary levels in male sheep.

SESSION VII

Clinical Andrology—B: Oral Presentations (37–42)

37. RESPONSE OF THE HUMAN TESTIS TO VASECTOMY. Martin Dym, Robert A. Newton, and Stuart S. Howards. *Department of Anatomy, Georgetown University, Washington, D.C.; Division of Urology, Newton-Wellesley Hospital, Newton, Maryland; and Department of Urology, University of Virginia, Charlottesville, Virginia.*

Testicular biopsies were obtained from seven patients undergoing vasovasostomy for reversal of male sterility secondary to vasectomy. The vasovasostomy surgery was carried out two months to seven years after the vasectomy. Nine biopsies were obtained from non-vasectomized control individuals. The testicular tissue was immediately fixed in 5% glutaraldehyde buffered with 0.2 M s-collidine, postfixed in 1.3% osmium tetroxide and prepared for subsequent light and electron microscopic examination. The interstitial tissue in the vasectomized men appeared normal. There was little evidence of hyalinization or fibrosis and the Leydig cells were of normal size and filled with common organelles similar to controls. Crystals of Reinke were evident. These observations are in accord with the reported normal circulating testosterone levels in vasectomized men. Although a number of the seminiferous tubules appeared normal, a significant proportion displayed a variety of abnormalities. These changes included a greatly thickened and infolded (wavy) basal lamina, a decrease in the number of mature spermatids, an in-

crease in the number of degenerating germ cells and an apparent lack of a tubule lumen. In four of the patients (four to seven years after vasectomy) spermatogenesis was clearly disrupted in portions of the biopsy. Ultrastructural observations confirmed and extended the light microscopic examination. There appeared to be an increase in the number of spermatids actively phagocytized by the Sertoli cells. We conclude that vasectomy may lead to alterations in the seminiferous epithelium. These patients will be monitored closely in attempts to correlate the observed state of the testicular biopsy with the re-establishment of fertility. (Supported in part by NIH grant HD-16227).

38. ALTERED TESTICULAR MORPHOLOGY IN VARICOCELE: A PRIMARY LESION IN SERTOLI CELLS. Don F. Cameron and Frank E. Snydle. *Department of Anatomy, University of Florida, Gainesville, Florida and Department of Obstetrics and Gynecology, Oakwood Hospital, Dearborn, Michigan.*

Testicular biopsies were obtained from 40 otherwise healthy men with diagnosed varicocele and processed for light (LM), scanning (SEM), and transmission electron microscopy (TEM). Biopsies of normal testes from two men with scrotal pain served as control. All varicocele biopsies displayed tubules with variably affected seminiferous epithelia. The basal testicular compartment (spermatogonia, preleptotene primary spermatocytes and basal Sertoli cell cytoplasm), and in general, all germ cell types present appeared morphologically normal. The adluminal testicular compartment, however, showed discrete ultrastructural and cell surface abnormalities limited primarily to the adluminal portion of malities limited primarily to the adluminal portion of Sertoli cell cytoplasm. Sertoli vacuolization and cytoplasmic degeneration left periluminal Golgi phase spermatids partially or completely divested of apical Sertoli cell cytoplasm. Sertoli cell junctional specializations were structurally abnormal and observed in conjunction with abnormal acrosome morphology and malorientation of acrosomal and cap phase spermatids. Tubule lumina contained a variable number of round spermatids. Results suggest that varicocele-associated testicular pathology is a function of the adluminal testicular compartment, wherein the site of primary lesion is the Sertoli cell. We suggest that apical Sertoli cytoplasmic degeneration and abnormal Sertoli-spermatid junctional complexes account for spermatid malorientation, disruption of normal spermiogenesis and excessive germ cell sloughing. We defined four stages of tubule pathology associated with varicocele and proposed a progression of adluminal testicular compartment dissolution precipitated by progressive deterioration of adluminal Sertoli cell cytoplasm.

39. EXOCRINE AND ENDOCRINE TESTICULAR FUNCTIONS IN HEALTHY GRANDFATHERS. U. Lammers, C. W. Freischem, E. J. Wickings, and E. Nieschlag. *Max-Planck Clinical Research Unit for Reproductive Medicine, F.R. Germany.*

Testicular functions were investigated in 23 grandfathers (60–88 years old, 67 ± 7.8 SD) ie men with fertility proven earlier in life. They were recruited from a non-patient population and lead an active life, most of them with a permanent partner. The grandfathers were compared with a group of 11 healthy men, 24 to 33 years old (27.7 ± 2.3) who had just fathered a child. There was no significant difference in sperm counts (120 ± 101 vs 106 ± 64 mill/ml) and sperm morphology (48 ± 9 vs 50 ± 12% normal) between the older and younger men. Sperm motility (50 ± 19 vs 73 ± 10%) and seminal fructose (1.7 ± 1.1 vs 3.2 ± 1.1 mg/ml), however, decreased with age. The fertilizing capacity of sperm as assessed in the HOP-test (heterologous ovum penetration test using zona pellucida free hamster eggs) did not decrease with age (percentage of penetrated eggs: 54 ± 19 vs 64 ± 17%). While the basal testosterone (T) and estradiol (E2) concentrations were not different between the older and younger group, the response to two days of hCG stimulation decreased significantly with age (T:1.4 ± 0.5 vs 1.9 ± 0.3 fold and E2: 2.7 ± 1.0 vs 3.8 ± 1.0 fold). This decrease was observed in older men whether they had frequent (at least once a week) or infrequent (less than every week) sexual activity. Although basal serum LH and FSH levels were higher in older men, the LH and FSH response to LHRH stimulation did not change with age. We conclude that sperm production and fertilizing capacity of sperm are not influenced by old age, at least not in healthy men with sustained sexual activity. Despite normal pituitary function a decrease in the endocrine capacity of the testes occurs as a characteristic of old age, which only few men can escape.

40. EFFECT OF ANDROGEN THERAPY ON FREE TESTOSTERONE AND TESTOSTERONE BINDING GLOBULIN IN HYPOGONADAL MALES. Stephen R. Plymate, J. M. Leonard, C. A. Paulsen, and B. L. Fariss. *Madigan Army Medical Center, Tacoma, Washington and USPHSH, Seattle, Washington.*

Free testosterone as determined by the interaction of testosterone (T) and testosterone binding globulin (TeBG) is an important determinant of androgen effect in the male. Since TeBG levels are often elevated in testicular insufficiency, it is important to determine whether TeBG declines into the normal range and free T becomes normal following androgen administration. In order to answer these questions, the following study was performed. Five normal males and six patients with Klinefelter's who had never been given testosterone were studied before and after the administration of testosterone enanthate, 100 mg every two weeks. Five patients with hypogonadotropic eunuchoidism were treated with HCG, 2000 U three times a week. Three months after administration of T or HCG, samples for T

and TeBG were drawn. TeBG was determined by dextrancoated charcoal saturation analysis, T was measured by radioimmunoassay, and free T was calculated by a modification of the formula of Perelman. Results are below:

	TeBG ng DHT/ml		T ng/ml		Free T pg/ml	
	Pre	Post	Pre	Post	Pre	Post
Klinefelter's	10.4 ± 3.0[2]	4.3 ± 0.8[2]	3.3 ± 0.7[3]	5.7 ± 0.2[3]	92 ± 25[2]	271 ± 7[2]
Hypogonadal Eunuchoidism	16.3 ± 5.0[1]	12.0 ± 5.0[1]	0.7 ± 0.2[4]	9.4 ± 3.6[4]	13.7 ± 6[5]	276 ± 16[5]
Normals	13.0 ± 1.8[3]	8.6 ± 1.6[3]	5.7 ± 0.9[4]	9.4 ± 1.8[4]	143 ± 41[4]	390 ± 94[4]

NS = [1] P < .01 = [2] P < .02 = [3] P < 0.05 = [4] P < .001 = [5]

The results demonstrate that the administration of to the Klinefelter's patient causes an appropriate decrease in TeBG and the normal free T. The response of TeBG also rules against androgen resistance in this syndrome. There was no significant difference in the TeBG levels in those patients treated with HCG, indicating a failure of suppression of TeBG by the androgen produced in response to HCG. The failure of this fall may be due to the concomitant rise in estradiol.

41. REDUCED FREQUENCY OF LH SECRETORY PULSES IN MEN WITH HYPERPROLACTINEMIA. Stephen J. Winters and Philip Troen. *Department of Medicine, Montefiore Hospital and the Clinical Research Unit, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.*

Hypogonadism is often found in men with prolactin (PRL) hypersecretion in whom low-normal serum gonadotropin concentrations with reduced circulating testosterone (T) levels suggest an abnormality in the hypothalamicpituitary unit. To probe the mechanism for this disordered gonadotropin secretion we studied LH concentrations in blood samples drawn every 20 min for 8 to 24 hr prior to and during chronic treatment with bromocriptine in four men with hyperprolactinemia, two of whom had intrasellar pituitary tumors and two had normal pituitary laminagrams and cranial CT scans. Basal serum PRL levels were 113–1200 ng/ml (normal, <20 ng/ml) and fell to 5.4 ± 5.2 ng/ml, (M ± SD), with treatment. Concomitantly, serum T levels rose from 221 ± 97 ng/dl to 490 ± 86 ng/dl ($P < 0.05$) (normal, 525 ± 95). TeBG binding capacity was uninfluenced by bromocriptine treatment, (0.66 ± 0.15 μg/dl vs 0.68 ± 0.12) (normal, 0.4–0.85). Serial determinations revealed that to reach peak serum T levels required up to four months of therapy. Mean serum LH levels increased from 34 ± 15 ng/ml to 72 ± 6 ng/dl ($P < 0.05$) (normal, 55 ± 14). In each subject the rise in serum LH was accompanied by an increased frequency of LH pulses and the mean interpulse interval fell from 260 ± 151 min to 130 ± 151 min ($P < 0.05$) (normal, 120 ± 32). There was no change in relative LH pulse height, 126 ± 100% vs 69 ± 57%. FSH levels rose from 103 ± 18 ng/ml to 142 ± 23 ng/ml (NS) (normal, 109 ± 39 ng/ml). After GnRH, 100 μg IV bolus, LH levels rose by 152 ± 58 ng/ml initially, and 246 ± 106 ng/ml during bromocriptine (NS). These data indicate that as serum PRL falls and T levels rise during bromocriptine treatment, mean serum LH concentrations increase as does the frequency of LH secretory episodes. Insofar as each LH pulse is believed to reflect a burst of GnRH, these data support the hypothesis that a major abnormality in hyperprolactinemic men is a reduction in episodic GnRH secretion.

42. PROSTATIC ACID PHOSPHATASE BY RADIOIMMUNOASSAY IN HUMAN SEMEN. Masaichi Kimura, Kaoru Takahashi, Hiroo Ishikawa, Shotaro Matsuda, Shiro Mitsukawa, and Seiichi Orikasa. *Department of Urology, Tohoku University School of Medicine, Seiryo-Machi, Sendai, Japan.*

The recently developed radioimmunoassay method for prostatic acid phosphatase (PAP) has been reported to be superior to the standard enzymatic technique in that it has a high specificity for PAP isoenzymes and for serum PAP detection in the early stage of prostatic cancer. The present study was designed to compare the radioimmunoassay and enzymatic analysis of PAP in seminal plasma in control cases and patients of male infertility. Radioimmunoassay of PAP was estimated using the kit of EIKEN (Japan) which was found to offer adequate sensitivity and precision. After liquefaction of the semen, the determination of the volume, sperm count and sperm motility of each sample were performed. Sperm cells were removed by centrifugation for 30 minutes at 4 C at 1,500 g and samples were stored at −20 C until radioimmunoassay and enzymatic analysis of PAP and the determination of fructose, zinc and prolactin. PAP levels were estimated to be as follows: normal (n = 21) 4,820 ± 566 μg/ml (mean ± SE), oligozospermia with sperm density of between 10 and 40 million/ml (n = 23) 3,791 ± 471 and severe oligozospermia with sperm density of less than 10 million/ml (n = 16) 4,673 ± 734. PAP levels of each group varied considerably, especially in severe oligozoospermia. We conclude that there is no direct correlation in the PAP levels determined by the two methods, PAP levels determined by radioimmunoassay in oligozoospermia with sperm density of between 10 and 40 million/ml show a tendency to decrease from the normal cases while enzymatic analysis shows no significant difference, and no correlation is observed between the PAP measured by the two methods and the seminal levels of fructose, zinc and prolactin, respectively.

Testing: Poster Presentations (43–55)

43. EVALUATION OF A SYSTEM FOR SPERMIOGRAMS. M. Diaz, F. Rivas, L. C.

Uribe, and M. Perez. *Clinica de Fertilidad, Hospital de Gineco-Obstetricia, Centro Medico de Occidente, Instituto Mexicano del Seguro Social, Guadalajara Jalisco, Mexico.*

Characteristics of semen have been evaluated by means of multiple criteria. Eliasson (Andrologie 1971; 3:49) has developed a system considering density, motile cells, degree of motility and sperm morphology. To evaluate the applicability of Eliasson's system for a Mexican population, single spermiograms from fifty fertile prevasectomized men were evaluated according to the Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Physical examination was normal in all cases. The mean ± SD for age was 33 ± 6 years, for marriage: 3 ± 7 years, for coital frequency: 3 ± 2/week, and for the number children: 4 ± 2. Normal scores (0–3) were found in 98% of the patients for spermatic density and mobility, in 100% for normal morphology. Normality (0–4) for all four parameters were found in 90% of patients. It is concluded that Eliasson system is applicable for this population. The "false negative" results could be explained because only one semen sample was obtained. It is also possible that some undetectable factor could impair some feature of the semen without affecting fertility.

44. EVALUATION OF SEMEN FROM EPIDIDYMIS FOR INSEMINATION AT THE TIME OF VASOVASOSTOMY. Edouard J. Servy and John B. Black. *Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, Georgia.*

Because of the doubt about the return of fertility in patients undergoing vasovasostomies, homologous artificial insemination (AIH) was attempted with the semen aspirated from the epididymis and the proximal portion of the vas deferens at the time of vasovasostomy in ten men. Two-tenths to two ml of semen per individual could be aspirated and were analysed. The concentration varied between 120 and 320 × 10^6/ml with a motility consistantly inferior to ten per cent, and many debris. In four out of ten, trichomonas vaginalis were found in large quantity, three of them presenting a positive Kibrick test (agglutinating antibodies) for a total of five positive Kibrick tests. Attention is drawn to the proliferation of entrapped trichomonas in the epididymis following vasectomy, the possible "adjuvant" inflammation effect of these parasites to begin antibody formation and the uselessness of AIH at the time of vasovasostomy.

45. A COMPARISON OF SPERM MOTILITY ASSESSMENT BY VIDEOMICROGRAPHY AND A "SUBJECTIVE" TECHNIQUE. Jeffrey Jenks, M. James Cosentino, and Abraham T. K. Cockett. *University of Rochester, School of Medicine and Dentistry, Rochester, New York.*

Twenty samples of human semen collected by masturbation were studied within one hour of ejaculation. Sperm motility for each sample was recorded by videomicrography and a "subjective" method currently used in our laboratory. The following information was obtained from the videomicrography studies: the mean linear velocity of speramtozoa, the percentage of motile spermatozoa (>25 μm/sec). The same samples were simultaneously assigned a motility score as manually determined by an experienced technician. The percentage of sperm falling into each of five motility grades (0–4) was determined for each specimen. The product of this percentage and the corresponding grade was then summed, thus giving the motility score for a given sample. The resulting data indicate strong correlations between the motility scores and each of the paramenters obtained with videomicrography (r = 0.87–0.94). The advantages and disadvantages of the two motility assessment techniques in a clinical setting will be discussed.

46. APPARENT GLYCOPROTEIN DEFICIENCY IN THE SEMINAL PLASMA OF ASTHENOZOOSPERMIC SEMEN. Walter J. Johnson, Rajasingam Jeyendran, Alan G. Hunter, and Mariano Perez-Pelaez. *University of Minnesota and Institute of Reproductive Medicine, Chicago, Illinois.*

Semen was collected from four normospermic males and eight asthenozoospermic males with infertile marriages. The asthenozoospermic semen was within normal levels for volume, leucocyte concentration, pH, sperm concentration and morphology but motility was low (58% normal vs 16% astheno). Upon liquification, the seminal plasma was removed by centrifugation, frozen and later analyzed at pH 8.3 by 7% polyacrylamide disc gel electrophoresis. Gels were stained with Coomassie Brilliant Blue for protein and periodic acid-Schiffs for glycoprotein. No obvious protein differences were seen between asthenozoospermic and normospermic seminal plasma. However, major differences were seen between the glycoproteins or normospermic semen and those in asthenozoospermic semen. Glycoprotein staining revealed two major glycoprotein bands (Rf 0.67 and 0.49) in normal seminal plasma. The band with the faster electrophoretic mobility stained moderate to strong both in the four normospermic semens and in the three asthenozoospermic semens from males with an identified clinical condition (large left varicocele, chronic prostatitis, bilateral hydrocele). However, this fast band was either very faint or absent in the five other asthenozoospermic samples. The staining intensity of the slower migrating glycoprotein band appeared to be associated with the pH of the seminal plasma rather than asthenozoospermia. In semen with pH between 7.43 and 7.73, the slow glycoprotein stained moderate to strong (7/7) while in semen with pH between 7.81 and 7.88, staining was negative or faint (5/5).

47. THE NEGLECTED LABORATORY TEST: THE SEMEN ANALYSIS. Augusto P. Chong,

Clifford Walters, and Shelley Weinrieb. *The Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Mt. Sinai Hospital, Hartford, Connecticut and the University of Connecticut Health Science Center, Farmington, Connecticut.*

Sixty-four laboratories from CT., MA. and CA. were involved in comparing the types of semen analysis offered. The collected data indicated a wide range of normal values given by these laboratories for each parameter considered in a semen analysis. Very few laboratories gave simple and precise instructions for collection of the specimens. The physical characteristics of the sample were poorly recorded. In many instances there were no records of collection time or the arrival time of the specimen at the laboratory. The technique for obtaining the concentration of the sample varied from laboratory to laboratory. Most laboratories did not report motility at time intervals after the initial percent motility reading. Very few laboratories gave a report on forward progression, and abnormal morphology was not broken down regarding the types involved. The following conclusions can be drawn from the analysis of data obtained in this study: 1. The majority of laboratories offering semen analysis had poor understanding and knowledge of male physiology and poorer knowledge of the spermatogenic process. 2. Very few laboratories update their normal values or follow the standards for semen analysis proposed by the World Health Organization. 3. An adequate assessment of the male factor can not be drawn based on such insufficient and incomplete information as reported by most laboratories for semen analysis. It is imperative that we recommend the implementation of adequate standards for such an important test, which is the basis upon which the clinician has to make the important decision of implicating the male partner in the couple's infertility problem or not. This recommendation should be directed to the American College of Pathologists since their members are the directors of private or hospital laboratories, in most cases.

48. EVALUATION OF *IN VITRO* EGG PENETRATION TESTS AND SEMEN ANALYSES ON FERTILE AND INFERTILE MEN: SIGNIFICANCE OF SPERM MOTILITY.

T. S. K. Chang, J. Walter Smyth, Don Vindivich, Steve Berry, and David Binko. *The Johns Hopkins Hospital, Baltimore, Maryland.*

The results of *in vitro* zona pellucida-free hamster egg penetration tests and semen analyses on eight fertile donors and twenty-six infertile patients were compared. The results of egg penetration tests, expressed as percent of zona-free eggs penetrated or penetration index (number of spermatozoa penetrating eggs/total number of eggs tested), were significantly higher for fertile men than for infertile patients ($P < 0.05$). For fertile donors, 20% to 100% of eggs were penetrated in 94% (17/18) of tests, and penetration indexes greater than 0.16 were obtained in 93% (14/15) of tests. In contrast, only 0% to 15% of zona-free eggs were penetrated, and penetration indexes of 0 to 0.16 were obtained, in 95% (38/40) of tests on infertile patients. Overall, 87% of fertile donors and 91% of infertile patients were correctly identified by the zona-free egg penetration test. When compared to semen analyses, the results of egg penetration tests were found best correlated with sperm motility, in particular, the quality of sperm motility (graded on a scale of 0–4). Samples with excellent grade 4 motility, whether assessed in semen, after removal of seminal plasma or immediately prior to insemination of zona-free eggs, consistently were better able to penetrate eggs than samples with grade 2 or 3 motility ($P < 0.05$). These studies demonstrate the effectiveness of egg penetration tests in the assessment of male fertility and infertility and stress the importance of the quality of sperm motility in sperm penetrating capacity. This study was supported by grants from the Marion I. and Henry J. Knott Foundation, the Andrew W. Mellon Foundation and the National Institutes of Health (HD-12762).

49. GLYCOSYLATED SEMINAL PROTEIN: A NEW TEST FOR THE EVALUATION OF MALE INFERTILITY.

F. T. Murray, T. Mehl, D. Cameron, J. Maurer, F. Rigall, and T. Merimee. *Departments of Medicine, Anatomy and Obstetrics and Gynecology, University of Florida, Gainesville, Florida.*

One hundred semen samples were analyzed for glycosylated seminal protein (GSP), a method used extensively to determine nonenzymatic adduction of hexose to a variety of proteins in diabetes mellitus. These samples consisted of 31 specimens from eight normal volunteers (NV) and 69 specimens from infertile males. This latter group was divided into several sets consisting of men with idiopathic oligospermia (IO) n = 7; prolactin pituitary tumors (PPT) n = 3; diabetes mellitus (DM); n = 1; prostatitus (P) n = 4; and male partners of infertile couples (MPIC), n = 54. Subset MPIC was divided in two groups; group I, GSP values greater than 2 SD of the normal mean and group II, normal GSP values. In addition to GSP, sperm count, motility, volume, and fructose were determined on most specimens. The table below illustrates mean values of GSP ± SD in each group.

	P	MPIC	
GSP	2.90	Group I	2.88 ± 0.69
nmol mg HMF protein	± 0.60	Group II	1.04 ± 0.60

	NV	IO	PPT	DM
GSP nmol mg HMF protein	1.36 ± 0.44	2.83 ± 0.86	3.29 ± 2.62	2.40 ± 1.51

GSP correlated inversely with dialyzed ($r = 0.337$, $N = 42$, $P < 0.05$) and undialyzed ($r = 0.528$, $N = 15$, $P < 0.05$) semen protein concentration, similarly, GSP correlated inversely with sperm count (millions/ml) ($r = 0.362$, $N = 38$, $P < 0.05$) and the ratio of protein to fructose ($r = 0.447$, $N = 21$, $P < 0.05$). We conclude that: GSP may be a useful parameter for the evaluation of male infertility and that the elevation in GSP is a direct result of alterations in seminal plasma proteins and fructose concentration.

50. SPECIMEN TO SPECIMEN VARIATION IN THE PARAMETERS OF SEMEN QUALITY OF FERTILE MEN David F. Katz, James W. Overstreet, Robert Pelfrey, Charleen Brazil, and Ernest L. Lewis. *Departments of Obstetrics and Gynecology, Urology, and Human Anatomy, School of Medicine, University of California, Davis, California.*

Relatively little is known about the specimen to specimen variability in the parameters that characterize human semen. Consequently, the extent to which a single ejaculate typifies the semen quality of an individual is not appreicated and the value of a single semen analysis may therefore be limited. To study this question, a longitudinal study was conducted of semen specimens from eight of the fertile donors in our therapeutic artificial insemination program. Eight to 46 specimens per man, collected at random over a ten-month period were studied. Sperm concentration was measured using a hemocytometer. The percentage of morphologically normal spermatozoa, the percentage of motile sperm and sperm swimming speeds were measured by analysis of video tapes. Considerable variability within individuals was found in both sperm concentration and total sperm numbers per ejaculate, the average coefficients of variation (cv) per man being 0.46 and 0.58, respectively. There was less variability in the percentage of motile spermatozoa (cv = 0.27). The least variability occurred in mean swimming speed (cv = 0.19) and in the percentage of morphologically normal cells (cv = 0.15). These results demonstrate that there is considerable variability in sperm concentration and total sperm numbers in successive ejaculates of individual fertile men. There is less variability in the percentage of motile spermatozoa, and especially in the sperm swimming speed and percentage of morphologically normal spermatozoa. Further analysis of within donor and among donor variability will also be presented.

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51. Abstract No. 51 has been withdrawn.

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52. SEMEN COLLECTION, EVALUATION AND FREEZING IN THE GIANT PANDA (Ailuropoda melanoleucus). C. C. Platz, Jr. *Texas A & M University, College Station, Texas.* D. E. Wildt and S. W. Seager. *National Institutes of Health, Poolesville, Maryland.* J. G. Howard and M. Bush. *National Zoological Gardens, Washington, D.C.*

Semen was obtained on four occasions from an adult Giant panda utilizing electrical stimulation produced from a 110 volt, AC, 60 Hz electric stimulator capable of 0–57 volts and 0–1.5 amps. The panda was anesthetized by an IM injection of CI744 (Parke-Davis). The stimulus was delivered via a rectal probe measuring 56.5 cm long, 4.5 cm diameter with 1.0 cm diameter × 15.0 cm long stainless steel electrodes positioned at 30°, 90° and 150°. The probe was lightly lubricated with a sterile lubricant prior to insertion 35 cm into the rectum. The semen was subjected to evaluation analysis and freeze preservation. Ejaculate volume, sperm count per ml of ejaculate and % sperm motility ranged from 2.3 to 3.6 m., 62 to 562 × 10^6 sperm and 45 to 83% motility, respectively. Spermatozoa were frozen using dry ice and a pellet method. Sperm recovery ranged from 40 to 55% motility and progressive motility range was 2.5 to 4.0, on a scale of 0 to 5.0, 5.0 being rapid forward progression. Three of the four collections were performed proximally to the estrus cycle of the female. The fourth collection was during the anestrous period and indications are that a seasonal variation in sperm production and testicular size exists in the species. Testicular volume was 53.9% of that measured during the estrus period of the female. Sperm dimensions recorded were: head length—5 μ, width—4.2 μ, midpiece length—7.2 μ , width—0.8 μ, flagellum length—39 μ, total length—51.2 microns. Abnormal morphologic forms of spermatozoa were observed in each ejaculate. These aberrant forms consisted primarily of bent or coiled flagella. Microscopic analysis of the thawed spermatozoa did not indicate any detectable gross damage due to the freezing process. No detectable decrease in sperm recovery or increase in aberrant forms were detected after storage in liquid nitrogen for 12 months.

53. CORRELATION OF SPERM AGGLUTINATION AND POSITIVE PROSTATIC EXPRESSATE. Eduardo R. Randrup and Joseph A. LaNasa, Jr. *Louisiana State University Medical Center, Department of Urology, New Orleans, Louisiana.*

Most instances of spermatozoal agglutination in semen samples might be attributed to either the presence of antisperm antibodies or of an infection of one of the male adnexal genital glands. The present paper is an attempt to correlate the presence of spermatozoal agglutination and positive prostatic expressate. Five-hundred consecutive semen samples were matched with the presence or absence of polymorphonuclear leukocyte clumping in the expressed prostatic secretion of the respective patients at the time they were obtained. Forty-two % of our semen studies presented

sperm agglutination and 58% did not. Of those with agglutination slightly more than half, or 54 percent, had WBC clumping in their EPS. Those free of sperm agglutination had positive EPS only in 36% of the cases. Sperm agglutination had a significantly higher incidence in patients with severe EPS leukocyte clumping (67%) than those with mild clumping (48%) or no clumping (35%). This will be defined further in the presentation.

54. CHARACTERISTICS OF HUMAN SEMEN SUBMITTED IN AN ARTIFICIAL INSEMINATION (AID) PROGRAM. R. H. Foote, L. S. Wix, M. C. Goldstein and T. R. Rounsaville. *Cornell University, Ithaca, New York.* R. Feldschuh and J. Feldschuh. *Idant Laboratories, New York, New York.*

Seminal characteristics of 1834 ejaculates from 15 semen donors over a period of five years in an AID program at Idant Laboratories were analyzed. The objectives were to establish some normal values for seminal characteristics associated with a recent sample of donors, donors of different ages and with different periods of abstinence. The ejaculate volume, sperm cell concentration per ml, total sperm cells per ejaculate and prefreeze and postthaw percentage of motile sperm cells (mean ± SE) for 1834 ejaculates were 3.16 ± .03 ml, 198 ± 1.9 × 10^6 sperm/ml, 643 ± 7.2 × 10^6 total sperm, 64 ± .1% prefreeze % motile and 44 ± .2% postthaw % motile. The range in means for corresponding characteristics of semen quality for donors with the lowest and highest average values were 1.6 to 4.6 ml, 125 to 299 × 10^6 sperm per ml, 435 to 971 × 10^6 sperm per ejaculate, 62 to 66% prefreeze sperm motility and 36 to 46% postthaw sperm motility. Semen characteristics by donors classified by age groups of <20, 21 to 25, 26 to 30, 31 to 35, 36 to 40 and >40 years were examined. There was little difference in the progressive motility of spermatozoa among age groups, either prefreeze (donor mean range of 63–64%) or postthaw (donor range 43–44%). However, total sperm per ejaculate (10^6) varied among the age groups as follows: 815 ± 42, 570 ± 17, 601 ± 14, 584 ± 13, 754 ± 16 and 737 ± 18. A majority of samples (1474) were obtained after three days of abstinence. Following 1, 2, 3, 4, 5 and 6 days of abstinence, total sperm per ejaculate (10^6) was 520 ± 29, 608 ± 51, 652 ± 8, 595 ± 15, 756 ± 74 and 940 ± 203. These data support the expected increase in number of sperm cells per ejaculate with longer periods of abstinence, but this must be interpreted cautiously because of the confounding between donors and intervals of abstinence.

55. DIRECTION OF SPERMATOZOON SWIMMING IN RESPONSE TO GRAVITY, FLUID FLOW AND SOLID WALLS. H. Winet and J Head. *Obstetrics and Gynecology, University of Southern California, Los Angeles, California and California Institute of Technology, Pasadena, California.*

Spermatozoa have been reported to exhibit positive geotaxis which may be the basis for some X and Y sperm separation techniques (Ericsson et al, 1973). They are also credited with negative rheotaxism which may be the basis for some motility evaluation techniques (Atherton, 1975). Observations of these taxes have been restricted to dead cells or post facto measurement of sperm accumulation patterns or frozen sections. In the present study live human spermatozoa were suspended in 1 mm wide angular cross-section tubes which were observed video-microscopically in vertical or horizontal orientation and in both steady flow and non-flow states. The direction of spermatozoon swimming was determined as a function not only of flow and orientation but also of distance from the tube wall. Rheotaxis was distinguished from drift by subtracting the later using tracer particle flow velocities. A strong tendency for motile spermatozoa to accumulate at the boundaries was noted for all tube orientations and flow rates; at least 50× more motile sperm are near the wall than at the tube axis. This radiad concentration is disrupted if flow is unsteady. Positive geotaxis was significant but not unilateral. About two spermatozoa swim upward for each three swimming downward in static as well as horizontally flowing fluid. Negative rheotaxis is exhibited by the majority of motile spermatozoa within 250 μm of the wall for tube axis velocities (TAVs) as slow as +5.8 μm/sec (+ = upward). This response is still present at TAVs up to at least +100 μm/sec but is restricted to a much smaller distance from the wall. The precise thicknesses of these annuli of negative reotaxism are also a function of spermatozoan velocity distributions and in the direction of flow—more negative rheotaxis in + flow. These results have implications for motile sperm suspensions in both *in vivo* and *in vitro* systems. (This study was supported by NIH grant #HD-51442-01).

Biochemistry: Poster Presentations (56–66).

56. AN ULTRASTRUCTURAL STUDY OF THE EARLY RESPONSE TO ACUTE HEAT INJURY IN THE RAT TESTIS. Richard V. Clark. *Developmental Endocrinology, NICHD, Bethesda, Maryland.*

This study describes the sequence of subcellular changes in the heat sensitive cells of the germinal epithelium of the rat testis following acute heat exposure. Testes were exposed to a water bath at 43 C for 15 minutes by scrotal immersion, removed at ½, 1, 1½, 5, 12, and 24 hours, fixed by vascular perfusion, and examined by electron microscopy. The germ cells which showed the earliest response to heat exposure were the late pachytene and dividing spermatocytes (Stages IX-XIV), and step 1 spermatids (Stage I). The ultrastructural injury pattern observed was: a) condensation of nuclear chromatin into a dense heterochromatin against the nuclear envelope, b) conformational changes in mitochondria consistent with deoxidation, c) redistribution of the form and location of endoplasmic reticulum, d) accumulation of dense material in the cytoplasm, and e)

apparent breakdown of the plasma membrane. These changes progressed rapidly and lead to pyknotic, dead cells by the five-hour sample. A few cell types, mid-pachytene spermatocytes (Stages VII-VIII) and step 15 spermatids (Stage I), did not show any evidence of injury until the 12 or 24 hour sample when they became pyknotic. The Sertoli cells phagocytosed 20 to 40% of the injured cells and released the rest into the tubule lumena. Within the Sertoli cells, vacuoles and mitochondria accumulated around the phagocytosed cells. The plasma membranes which formed the phagosome underwent apparent dissolution at early sample periods, but at 24 hours, a single continuous phagosome membrane was found. The Sertoli cells did not show evidence of injury except those in Stage I which developed large vacuoles filled with multiple dead cells at the 24 hour sample. No evidence of injury was observed in spermatogonia or Leydig cells. These results detail the intracellular events leading to cell death after acute heat exposure and indicate that complex cell interactions, especially among Sertoli cells, are involved in the response to heat injury.

57. FRAGMENTATION OF EPITHELIAL CHROMATIN FROM GUINEA PIG SEMINAL VESICLE BY ENZYME DIGESTION.

Daniel G. Remick, Jr. and Carlo M. Veneziale. *Mayo Medical School, Rochester, Minnesota.*

Micrococcal nuclease digestion of nuclei from guinea pig seminal vesicles in 0.1 mM $MgCl_2$ produced a mixture of sub-nucleosome fragments, nucleosomes, and polynucleosomes. Digestion proceeded more quickly when the reaction was buffered with 10 mM Tris-HCl, pH 7.4 Increasing the ionic strength to 10 mM NaCl or 3 m M $MgCl_2$ also increased the rate of digestion. There was no apparent difference in digestion products or kinetics of digestion between nuclei from intact animals or those castrated 24 hours previously. A_{260} measurements of sucrose gradient fractions revealed three predominant peaks; one of submononucleosome particles, an 11S peak of nucleosomes and a third broad peak composed of polynucleosomes. Electron microscopy of chromatin fragments from the polynucleosome region, employing a Pt-Pd mist procedure, disclosed the characteristic beads-on-a-string appearance. The DNA fragments size was determined using SDS-6% slab gel polyacrylamide electrophoresis with I X 174 cut with Hae III molecular weight markers. The digestion products contained DNA of the following sizes: 89 to 99 base pairs (bp), 133 to 154 bp, 196 to 267 bp, 377 to 492 bp, 610 to 680 bp. Electrophoresis of acid-extracted proteins revealed bands corresponding to the four major histone groups. Our results demonstrate that chromatin from guinea pig seminal vesicles is organized in the same manner as has been described for other tissues and that the removal of endogenous androgens, by castration 24 hours previously, does not grossly alter that organization.

58. MEMBRANE-BOUND INTRANUCLEAR INCLUSIONS IN LEYDIG CELLS OF CHINESE HAMSTER TESTIS.

A. F. Payer and T. A. Parkening. *Department of Anatomy, The University of Texas Medical Branch, Galveston, Texas.*

Ultrastructural investigations on Leydig cells of the Chinese hamster revealed membrane-bound intranuclear inclusions not previously described as a common morphological feature of Leydig cells in mammalian testes. Nuclei of Leydig cells from C. hamsters were examined at 1 day, 19 days, 1, 1.5, 5, 6, 17, 19.5, 23, and 30 months of age. The testes were perfused with 2% glutaraldehyde-2% paraformaldehyde in 0.1 M phosphate buffer and post fixed in 1% OsO_4 or 1% OsO_4-2.5% $K_4Fe(Cn)_6 \cdot 3H_2O$ in 0.1 M phosphate buffer. Some tissue was similarly processed with cacodylate buffered fixatives and incubated for the trimetaphosphatase (TMPase) reaction to study the lysosomal system. Random areas from each testes were examined ultrastructurally. Intranuclear inclusions were not found in the 1 day to 1.5 month age groups, but were present in sexually mature and aged animals. Some groups of Leydig cells did not contain any intranuclear inclusions while other groups had inclusions in a few or all cells. Some inclusions occupied a large area of cross-sectioned nuclei while others were only visible with electron microscopy. Serial sections showed that the inclusions were derived from nuclear invaginations of cytoplasm which became enclosed within the nucleo-plasm by a single unit membrane. The inclusions usually had an amorphous content, occasionally contained cytoplasmic organelles and rarely had electron dense globular units. The inclusions were found in Leydig cells with either round or irregular-shaped nuclei and the cytoplasmic morphology was always normal. Cytochemical studies showed the TMPase localization in vesicles within the nuclear invaginations and the intranuclear inclusions. These studies indicate that the intranuclear inclusions of C. hamster Leydig cells originated from cytoplasmic material that became enclosed within the nucleoplasm, contained vesicles that were TMPase positive, were only found in sexually mature and aged males, and increased in numbers with age.

59. SYNTHESIS OF STAGE SPECIFIC PROTEIN MARKERS IN PACHYTENE PRIMARY SPERMATOCYTES AND ROUND SPERMATIDS FROM MURINE TESTIS.

Leslie Stern, Bert Gold, and Norman B. Hecht. *Department of Biology, Tufts University, Medford, Massachusetts.*

Analysis of the populations of messenger RNA from several testicular cell types by cell-free translation suggests stage-specific differences in the polypeptides encoded (Gold et al, 1981). To ascertain whether a selective utilization of messenger RNAs occurs *in vivo* during

spermatogenesis, the proteins synthesized in mouse pachytene primary spermatocytes and round spermatids were solubilized and analyzed by one- and two-dimensional gel electrophoresis. Following intratesticular injection of ^{35}S methionine or ^{3}H leucine, populations of pachytene primary spermatocytes and round spermatids were purified by unit gravity sedimentation and density gradient centrifugation through Percoll to >90% purity. Although the majority of proteins detected following a radiolabelling interval of 2 to 24 hours were synthesized in both pachytene primary spermatocytes and round spermatids, numerous specific differences were found in the two-cell types. For example, polypeptides that appeared either markedly enriched or unique to pachytene primary spermatocytes included those of estimated molecular weight 71,000, 39,000, 33,000, and 19,000 daltons while round spermatids synthesized high levels (relative to pachytene primary spermatocytes) of polypeptides with estimated weights of 74,000, 49,000, 45,000, and 43,000 daltons. A more limited number of differences between the polypeptide composition of the two cell types was observed when total cellular proteins were detected with Coomassie brilliant blue R. These studies demonstrate differential gene expression occurs *in vivo* during mammalian spermatogenesis and provide evidence for the synthesis of stage specific proteins for two representative testicular cell types. (Supported by N.I.H. Grant GM 29224).

60. SYNTHESIS OF GLYCERYLPHOSPHORYLCHOLINE (GPC) FROM PHOSPHATIDYLCHOLINE (PTC) *IN VITRO* BY RAT EPIDIDYMAL SPERM AND PRINCIPAL CELLS.

Christina Y. Wang, David A. Chapman and Gary J. Killian. *Department of Biological Sciences, Kent State University, Kent, Ohio.*

Rat caput, corpus and cauda epididymal sperm were incubated with ^{14}C-phosphatidylcholine (^{14}C-PTC) and the uptake rates were determined. All sperm types bound ^{14}C-PTC (nmoles/10^{10} sperm) within 30 sec and peaked at 8 min (117 ± 23 for caput, 74 ± 11 for corpus and 39 ± 11 for cauda). For the remainder of the 32 min incubation there was a gradual decline in the amount of ^{14}C-PTC associated with sperm. Throughout the entire incubation caput sperm had the greatest affinity for ^{14}C-PTC followed by corpus and cauda sperm. Since significant amounts of ^{14}C-glycerylphosphorylcholine (^{14}C-GPC) were found in caput, corpus and cauda sperm (9.8 ± 1, 7 ± 1, 3 ± .4 nmoles/10^{10} sperm) and their corresponding supernatants (26 ± 3.4, 22 ± .4, 15 ± 2.5 nmoles/10^{10} sperm) after 2 hr, it is suggested that epididymal sperm convert ^{14}C-PTC to ^{14}C-GPC. Caput principal cells synthesized and secreted ^{14}C-GPC (nmoles/10^{10} cells) when incubated with either free ^{14}C-PTC (86 ± 6) or ^{14}C-PTC-labeled sperm (82 ± 7). The total ^{14}C-GPC (nmoles/10^{10} sperm) produced after 2 hr from the co-incubation of principal cells with ^{14}C-PTC-labeled caput sperm (97 ± 5) was comparable to the total amount predicted by summing the ^{14}C-GPC synthesized from each incubation of sperm (13 ± 1) or principal cells (86 ± 6) alone. Nevertheless, in single incubations 58% of the ^{14}C-GPC produced was secreted while in co-incubations 71% of the ^{14}C-GPC produced was secreted; ^{14}C-GPC secretion apparantly was stimulated by combining sperm and principal cells. It was concluded that high GPC levels in the epididymal fluid are sustained partly by sperm and principal cells by the degradation of PTC. Preliminary studies suggest that the synthesis rate of ^{14}C-GPC by principal cells incubated with ^{14}C-choline is 10× that obtained using ^{14}C-PTC precursor, however, the synthesis rate of ^{14}C-GPC by epididymal sperm is 10× greater using ^{14}C-PTC than ^{14}C-choline substrate. (Supported by HD 10827).

61. INVESTIGATION OF ANDROGEN-DEPENDENT mRNA FROM RAT VENTRAL PROSTATE USING CLONED cDNA.

Don B. Carter, Koji Yamada and Stephen E. Harris. *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.*

The androgen-dependence of two mRNAs from rat ventral prostate coding for a 20,000 and a 11,000 dalton translation product has been investigated using complementary DNA cloned in the bacterial plasmid PBR322. One of the cloned insert DNAs from a recombinant plasmid, C-27, arrests the *in vitro* translation of C_2 (Peeters, et al. JBC 1980; 244:7017–7023). The other cloned insert DNA arrests the translation of a glycoprotein 20,000 daltons in size, with unknown function. The quantities of mRNA coding for the 20,000 and 11,000 dalton translation product were determined by hybridization of ^{32}p-labeled inserts to filter bound total RNA or poly(A^+)-mRNA. Castration caused a decline in both mRNAs of 250-fold over eight days. Stimulation with androgen of five week castrates restored the mRNA levels to 17% of intact for the 20,000 dalton translation product and 31% of intact for the 11,000 dalton translation product. The quantity of the two mRNA's found in the lateral poly (A^+)-mRNA was about 1/10 that of the ventral level and the mRNA's were not detectable in the dorsal prostrate, seminal vesicle or human prostate poly (A^+)-mRNA populations. RNA from the ventral prostates of animals 10 days and 21 days old contained mature levels of complementary sequences, suggesting a form of developmental post-transcriptional regulation for synthesis of the polypeptides which are not synthesized in mature quantities at this stage of development (Heyns et al. Endocrinology 1978; 103:1090–1095; Kistler et al. Proc Natl Acad Sci USA 1978; 737–741). A genomic fragment containing sequence information for the 20,000 dalton translation product has been isolated from a Charon 4A rat gene library. Subcloning of this fragment in PBR325 is now in progress.

62. A PRELIMINARY ELECTRON MICROSCOPIC SURVEY OF THE EXFOLIATIVE CYTOLOGY OF HUMAN SEMEN. Margaret L. Couture and Matthew Freund. *Department of Obstetrics and Gynecology, New Jersey School of Osteopathic Medicine, Camden, New Jersey.* Lonnie Russell, *Department of Physiology, Southern Illinois University Carbondale, Illinois.*

Previous studies in this laboratory characterized the normal exfoliative cytology of human semen by light microscopy. This study was undertaken to characterize these exfoliated cells by electron microscopy. Semen specimens were obtained from three vasectomized and three nonvasectomized men and routinely processed for electron microscopy. Vasectomized men were used to eliminate the possibility of confusing exfoliated genitourinary tract cells with immature germ cells. Histochemical localization of acid phosphatase was made, using B-glycerophosphate as substrate. In a survey of approximately 100 cells, the following exfoliated cell types were observed: a prostate cell, tentatively labelled as such because of its characteristic morphology and high localization of acid phosphatase; a cell of similar appearance without high acid phosphatase localization, which was the prevalent cell type; a squamous cell, most probably of urethral origin, which was the second most prevalent cell type; a seminal vesicle cell, tentatively labelled as such because of its characteristic electron dense secretory vacuoles containing lipofuscin granules; a basal cell, most probably originating either from the prostate or seminal vesicle; a binucleate cell of undetermined origin. Neutrophils were the most prevalent white blood cell observed and were the third most prevalent cell type. A small number of macrophages were observed, with those in vasectomized semen tending to have more phagocytic inclusions than those in nonvasectomized semen.

63. CHANGES IN SURFACE COMPONENTS OF CHIMPANZEE SPERMATOZOA AT EJACULATION. L. G. Young, K. G. Gould, and B. T. Hinton. *Department of Physiology and Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia and Department of Urology, University of Virginia School of Medicine, Charlottesville, Virginia.*

To evaluate changes in plasma membrane components of chimpanzee spermatozoa at ejaculation, externally oriented proteins/glycoproteins on the surface of chimpanzee vas deferens and ejaculated spermatozoa were labeled by enzymatic iodination with lactoperoxidase and ^{125}I-NaI. SDS-7.5% PAGE of labeled vas deferens spermatozoa surfaces resolved three components with approximate molecular weights of 81 to 83k, 65 to 68k and 44 to 46k daltons. SDS-7.5% PAGE of labeled ejaculated spermatozoa surfaces resolved four components with approximate molecular weights of 70 to 74k, 46 to 52k, 24 to 26k and 16 to 18k daltons, one of which comigrated with a labeled vas deferens spermatozoa surface component. Of the remaining components, two were associated exclusively with vas deferens spermatozoa and three were associated exclusively with ejaculated spermatozoa. To identify components adsorbed to the surface of vas deferens spermatozoa from adnexal secretions at ejaculation, chimpanzee seminal plasma was labeled and analyzed by the same procedure. Electrophoresis of seminal plasma resolved three labeled proteins/glycoproteins with approximate molecular weights of 62 to 64k, 25 to 26k and 16 to 17k daltons. Two of the components labeled in seminal plasma comigrated with components labeled on the surface of ejaculated chimpanzee spermatozoa and one was unique to seminal plasma. These observations indicate that, at ejaculation, specific macromolecular components are lost, masked or added to the surface of chimpanzee vas deferens spermatozoa. (Supported by BRSG RR05364 to EUSM, Ford Foundation Grant 690-0645B and NIH Grant RR00165).

64. DIFFERENCES IN GLYCOSIDASE ACTIVITY AMONG CELL TYPES COMPRISING THE RAT EPIDIDYMIS. Gary J. Killian and David A. Chapman. *Department of Biological Sciences, Kent State University, Kent, Ohio.*

Populations of principal cells, basal cells and fibroblasts were isolated by centrifugal elutriation from enzymatically dispersed caput and corpus epididymidis of the rat. Levels of activity of β-galactosidase, β-N-acetylglucosaminidase, β-N-acetylgalactosaminidase and β-glucosidase were determined fluorometrically for each cell population after its incubation with 4-methylum-belliferyl substrate. Maximum levels of activity (μ moles of 4-methylumbelliferone liberated/10^{10} cells/hr) of β-galactosidase (19.3 ± 1.6), β-N-acetylglucosaminidase (58.9 ± 9.5) and β-N-acetylgalactosaminidase (39.2 ± 8.9) were detected in corpus principal cells and were approximately 2-, 3- and 4-fold greater than corresponding enzyme activity detected in caput principal cells. Corpus/caput principal activity ratios for these enzymes remained constant for determinations done at alternate temperatures, pH's and substrate concentrations, suggesting that similar enzyme forms were present in the caput and corpus epididymidis. Principal cells from both the caput and corpus showed significantly more activity for β-galactosidase (7–12×), β-N-acetylglucosaminidase (10–29×) and β-N-acetylgalactosaminidase (8–90×) than that of basal cells and fibroblasts from the same region. Levels of β-glucosidase were similar in caput and corpus principal cells, and generally greater than levels in basal cells and fibroblasts. For epididymal sperm, levels of glycosidase activity generally increased from caput to corpus, but this trend was significant only for β-N-acetylglucosaminidase. Activities of β-N-ace-

tylglucosaminidase and β-N-acetylgalactosaminidase were significantly greater in corpus sperm than in cauda sperm. The peak levels of glycosidase activity associated with the corpus epididymidis may indicate that this region is a major site for modification of sperm glycoproteins during sperm transit. (Supported by HD-10827).

65. CHANGES IN THE BIOCHEMICAL COMPONENTS DURING LIQUEFACTION OF HUMAN SEMEN. Asok K. Bhattacharyya, Arabinda Mondal, and Subir R. Sarkar. *Department of Biochemistry, University of Calcutta, Calcutta, India.*

Human semen coagulates during ejaculation and normally liquefies in approximately 5 to 20 minutes. Almost nothing is known about the changes that occur during liquefaction. In the present study, we monitored the gradual changes of several biochemical parameters during liquefaction of normal, oligo, acute oligo, astheno, necro, azoo and vasectomised human semen using the bag (20 μm mesh size) method of Tauber et al (Fertil Steril 1980; 33:567). Selected 150 subjects (age: 18–45 yrs) were included in our study and samples were analysed within 3 minutes of ejaculation. Proteins were released from the coagulum at an average rate of 1.5 mg/ml semen/min during liquefaction. However, after complete liquefaction, protein contents decreased slowly with a rate of 0.11 mg/ml semen/min. Free amino acid level increased almost linearly up to 60 minutes of incubation with an average rate of 48 μg/ml semen/min. A significant increase in the release rate of free phosphorus (3.6 μg/ml semen/min) from the coagulum was also observed. Reducing sugar level increased both during and after liquefaction stages with an average rate of 75 μg and 15 μg/ml semen/min respectively. Citric acid and acid phosphatase levels remained relatively constant through out the entire process of liquefaction. During the first five minutes of liquefaction, a sharp disappearance of both alkaline phosphatase and arylamidase activities was noted, whereas neutral proteinase showed a gradual increase in its activity during liquefaction. The results indicate that a relationship appears to exist between the increase in proteinase activity of the seminal coagulum and the rate of its liquefaction. (Supported by Grants from ICMR and WHO).

66. ^{31}P-NMR ANALYSIS OF A MURINE LEYDIG CELL TUMOR. Michael H. Melner, Thian C. Ng, William T. Evanochko, Narinda Gill Kumar, Jerry D. Glickson, and David Puett. *Vanderbilt University, Nashville, Tennessee and The University of Alabama, Birmingham, Alabama.*

^{31}P-NMR was evaluated as a potential tool in studying hormonal induction and promotion of tumor growth utilizing the transplantable murine Leydig cell tumor M5480A. The levels of various phosphorous metabolites were measured *in vivo* in subcutaneously implanted M5480A tumors with the use of a specially designed probe equipped with a surface coil. Fourier transform NMR spectra were measured on a Bruker CXP-200/300 spectrometer. Experiments were performed at 80.96 MHz employing a 70° flip angle (25 μsec), a 3-second pulse delay, and 4096 data points. Methylene diphosphonate placed in a capillary tube on the opposite side of the coil served as a secondary chemical shift reference (−16.3 ppm). Chemical shifts are reported relative to 85% inorganic orthophosphate as standard. Mice were analyzed prior to hormone treatment and then subsequent to intraperitoneal injection of human GH which, in addition to somatotropic actions, exhibits prolactin-like effects in these tumors and testis. Spectra obtained prior to GH stimulation revealed an acidic pH (6.97) within the tumors. By comparison with published spectra, tentative resonance assignments were made. These spectra revealed prominent resonances for sugar phosphates (6.799 ppm), inorganic phosphate (4.77 ppm), ATPγ and ADPβ (−2.467 ppm), ATPα and ADPα (−7.731 ppm), and ATPβ (−15.9 ppm). At 6 hr following injection with GH, there was a dramatic increase in phosphocreatine levels (0 ppm). These data demonstrate changes in phosphorous metabolites following hormonal stimulation and suggest that ^{31}P-NMR analysis of endocrine tumors is a noninvasive and potentially useful tool in the study of hormonal induction and promotion of tumor growth. (Supported by AM15838, CA13148, and CA09131).

Clinical Andrology—A: Poster Presentations (67–72)

67. INCREASED LEVELS OF PESTICIDES IN THE LEFT TESTIS IN MEN WITH VARICOCELE. Grzegorz A. Szymczynski and Stefan M. Waliszewski. *Department of Andrology, Medical School, Jackowskiego 41, Poznan, Poland.*

Biopsy material from both testicles obtained during surgery from seven patients with varicocele was studied. In all the cases semen analytic values showed teratozoospermia. Content of pesticides: HCB, DDE and BHC-isomers were analyzed using gas liquid chromatography with ^{63}Ni electron capture detector (GLC-ECD) described in detail in previous report (Szymchyaski, 1981). The average values obtained (Table) clearly showed the increased levels of persistent pesticides in the left testis. TABLE. Mean levels of chlorinated pesticides in left and right testicles (14 samples). Data in ug/g of testicular tissue.

	HCB	alpha	gamma	beta	delta	epsilon	DDE
Testis		−BHC	−BHC	−BHC	−BHC	−BHC	
Right	.014	.010	.017	.079	.032	.060	.065
Left	.018	.014	.028	.122	.033	.055	.078

Of the pesticides under study, most persistent are DDE, HCB, beta-BHC and gamma/BHC. Increased values of these pollutants were noted in the left gonad. Evident lateralization of these persistent toxic compounds throws a new light on the etiopathogenesis of the teratozoospermia in patients with varicocele.

Szymczynski, G. A. and Waliszewski S. M. Selected chlorinated pesticides in human testicles. Proceedings of the INSERM International Symposium on Human Fertility Factors (with emphasis on the male). Cargese, September 21–23, 1981 (in press).

68. HISTOLOGICAL CHARACTERIZATION OF THE EPIDIDYMIS AND TESTIS IN PATIENTS WITH BILATERAL VAS DEFERENS AGENESIS.

Jean L. Fourcroy, Mary C. Bibro and Paul J. Christenson. *National Naval Medical Center, Bethesda, Maryland.*

It has been assumed that spermatozoa from either testis or caput epididymis are incapable of fertilizing ova, but recent reports would suggest otherwise (Kelami, 1981). In a recent review it was found that agenesis of the vas deferens and/or epididymis approaches 1% in infertile patients and 95% of patients with bilateral agenesis retain at least part of the proximal epididymis (caput or initial segment) (Christensen, 1981) and are, therefore, potentially fertile patients. The caput and efferent ductules may share a common embryologic origin differing from the rest of the epididymis (Marshall, 1979). We have histologically evaluated several patients with bilateral agenesis with caput remaining on surgical exploration. Biopsy of the epididymis was compatible with the characteristics of cells primarily of efferent ductules including ciliated cells, stereocilia, pseudostratified epithelium with supranuclear secretory and pigment granules and irregular luminal surfaces. Enzymatic characteristics of these cells will be presented including phosphatase, NAD diaphorase and non specific esterase histochemical assays. All stages of spermatogenesis are observed in the testis, but a decrease in late spermatid stages is noted. The characteristics of the epididymis present in these three patients with bilateral agenesis again suggests a defect limited to the mesonephric duct-derived structures alone, and a pattern comparable to known animal models. Further studies are needed to evaluate spermatozoa maturation.

69. THERMOSTRIP® DETECTION OF VARICOCELE.

F. Comhaire and L. de Thibault de Boesinghe. *Department of Internal Medicine and Nuclear Medicine, State University Hospital, Ghent, Belgium.*

Careful scrotal palpation yields inconclusive findings in 11%, and false negative results in 20% of subfertile men nevertheless presenting pathological inversion of blood flow in the internal spermatic vein(s). Since interruption of this reflux may improve semen quality, eventually resulting in repair of fertility, subclinical reflux should be searched for by means of special techniques. Doppler blood flow measurement using a bi-directional device, and thermography are suitable screening methods, the former being however less reliable than the latter. The performance of telethermography necessitates expensive equipment, whereas contact thermography using rigid plates is difficult because of the anatomy of the male genital region. Contact thermography using flexible strips (Thermostrip®, kindly provided by Bayer) is a cheap and easy office-method for the detection of scrotal hyperthermia. A set of three strips (34, 33, 32 C) allows full examination of scrotal skin temperature. Results of contact- and telethermography were found to be identical in over 100 cases examined in parallel. False positive thermographic findings occurred in 8% of cases, the incidence of false negative results is currently under investigation. (Supported by WHO Special Programme of Research, Development and Research Training in Human Reproduction. Project nr. 79902).

70. A REEVALUATION OF DAILY SPERM OUTPUT IN HUMANS.

Larry Johnson. *Cell Biology, The University of Texas Health Science Center at Dallas, Dallas, Texas.*

Since daily sperm output (DSO) has been estimated (Freund, 1963) at half of a recent estimate (Johnson, et al, 1981) of daily sperm production (DSP), DSO was reevaluated and urinary loss of sperm was determined in nine men aged 26 to 38 years. Each of five daily ejaculates was collected into graduated, 50-ml vials and immediately fixed by the addition of ~10 ml 2% glutaraldehyde in 0.1M sodium cacodylate. The volume of the fixed semen was increased to 50 ml by the addition of homogenizing fluid (150mM NaCl, 0.05% (v/v) Triton X-100, and 3.8mM NaN_3) to the collection vials. An additional 100 mls of homogenizing fluid were used to rinse the collection vials into the container of a Waring blender. After 2 minutes of homogenization, clumps of fixed semen were dispersed, and a uniform suspension of sperm was produced. The total number of sperm per ejaculate was equal to the product of 150 mls and the concentration of sperm in the homogenate determined by phase-contrast cytometry. To estimate DSO for each individual, the total number of sperm per ejaculate in each of the last three of five daily ejaculates was averaged. Based on preliminary data from two men, two daily ejaculates were sufficient to stabilize extragonadal sperm reserves following five days of sexual rest. Likewise, 48 hours with daily ejaculations were sufficient to stabilize sperm reserves following depletion by three ejaculations per day. To estimate the number of sperm being voided in the urine, urine was collected for 24 hours between the fourth and fifth daily ejaculates. Sperm were found in the urine of only three men, and numbers ranged from 0.1 to 1.5 $\times 10^5$ sperm. The total number of sperm per daily ejaculate for the last three of five days was 139 $\pm$ 21 $\times 10^6$, 174 $\pm$ 29 $\times 10^6$, and 187 $\pm$ 50 $\times 10^6$ sperm, respectively. DSO based on these three

ejaculates was 166 ± 29 × 10^6 sperm with a range of 84 ± 7 × 10^6 to 371 ± 84 × 10^6 sperm. Hence, few sperm are voided in the urine of men ejaculating daily, and DSO significantly exceeds previous estimates and approaches DSP (195 × 10^6) of a similar age group of men.

71. SPONTANEOUS & OXYMETHOLONE-INDUCED GYNECOMASTIA. V. Cortés-Gallegos, G. Castañeda, R. Alonso, E. Pérez-Pastén, V. Reyes-Lugo, C. Barrón, L. Mondragón, and S. Villalpando. *División de Endocrinología-Reproducción y Hospital de Pediatría, UIB, CMN, IMSS, México City.*

Gynecomastia is common and physiological in boys at puberty. Several drugs—spironolactone, cimetidine, cyclophosphamide—have been associated to it. Oxymetholone (OXY), an anabolic steroid used in aplastic anemia was associated with gynecomastia. The present report concerns a possible mechanism. The subjects studied were: 1. pubertal spontaneous gynecomastia (n = 9, ages: 12–15 yrs; 2. OXY-Gynecomastia (under 100–125 mg/d/2–7 yrs, n = 5, ages: 15–17) and 3. healthy young volunteers, n = 40, at stage I1–V of Tanner). Blood samples were obtained (a total of four, at 20 min. interval each for 1. and 2. and individual samples for 3.) to measure FSH, LH, PRL (ng/ml), T, DHT, E-2 and E-1 (pg/ml). No difference was found in steroids and gonadotropins/PRL, when groups 1 and 2 were compared on a stage matched control basis; except for T (>2 SD in 5/9 subjects of group 1) and DHT (<2 SD in all of 1 and 3). The T: DHT ratio varied from 5.0 to 15.4 in 1 and from 0.42 to 2.24 in 3. PRL, T, E-2 and E-1 values of group 2 were similar to those of group 3, but different in LH: 60 ± 24 vs 80 ± 17, $P < 0.01$; FSH: 139 ± 52 vs 214 ± 67, $P < 0.01$ and DHT: 399 ± 264 vs 3022 ± 984, $P < 0.001$). One could consider but would not necessary have to postulate: 1. in spontaneous gynecomastia may exist an enzimatic blockade of 5α reductase; 2. the T:DHT decrease may favour the estrogen action for the progression of breast enlargement; 3. OXY might be aromatizable, inducing this local disorder, and 4. OXY induces a secondary hypogonadism with normal T and restrictive formation of DHT.

72. INDUCTION OF SPERMATOGENESIS IN HYPOGONADOTROPHIC HYPOGONADISM BY PULSATILE ADMINISTRATION OF LHRH E. J. Keogh, S. A. Mallal, S. C. McColm, A. G. Dunn, T. Marshall, J. Attikiouzel, R. Hague and N. Bateson. *University of Western Australia, Barnsley Regional Hospital, United Kingdom.*

Three azoospermic men presented with infertility and incomplete sexual maturation. Patient a (28 years), had Kallmann's syndrome; b (22 years), idiopathic hypogonadotrophic hypogonadism and c (26 years), developed diabetes insipidus and hypogonadism following head trauma. Basal gonadotrophins and testosterone were low and failed to increase during LHRH infusion studies (1 μg/min for 4 hours). LHRH was administered in a pulsatile fashion from a Graseby-Dynamics MS16 syringe driver at 90 to 160 μg/day. This battery operated device (180 g) which was worn in a shoulder holster, was modified by incorporation of an integrated circuit to deliver (0.28 ml of LHRH solution over 3 min every hour via a 25 g scalp vein needle to the subcutaneous tissue of the anterior abdominal wall. Within two weeks of commencing therapy, patients a and c noted improved libido and strength which was paralleled by increments in both gonadotrophins and testosterone. Secondary sex hair growth increased and they were able to ejaculate. After 37, 8 and 14 weeks sperm concentrations increased reaching 2, 120 and 50 × 10^6 ml^{-1}. The wife of patient c conceived when his sperm count was 3 × 10^6 ml^{-1} and was subsequently delivered of a normal boy. This patient c was then given human chorionic gonadotrophin, 1,500 U/week for 11 weeks during which his sperm count remained in the range 30 to 95 × 10^6 ml^{-1} ($\bar{x}$ 50) while sperm was stored. Following cessation of hCG the sperm count fell. Antibodies to LHRH were not detected. Monthly LHRH infusion tests showed a resumption of the normal adult pattern of gonadotrophin secretion. Conclusion: The portable syringe driver is a practicable means of administering low dose LHRH in a pulsatile fashion which simulates the normal mode of LH stimulation. The efficacy of this regime is reflected in the prompt increase in testosterone levels and sperm production.

Hormonal Regulation: Poster Presentations (73–81)

73. STEROIDOGENIC RESPONSE TO hCG UNDER CONDITIONS KNOWN TO DEPLETE TESTICULAR hCG BINDING SITES. A. Bartke, A. Amador, M. L. Martinez, and M. H. Stallings. *Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, Texas.*

We have previously shown that hCG injected subcutaneously in mice or hamsters, in doses equal to or greater than 0.3 IU/g bwt, is capable of depleting the levels of testicular hCG binding sites. This study was undertaken to determine whether the loss of hCG binding sites inhibits the steroidogenic response to hCG. Normal and dwarf mice were used. The dwarfs differ from the normals in being deficient in PRL, GH and TSH, and in having lower levels of testicular hCG binding sites. Both types of mice were injected with either saline or different doses of hCG (identical to those used in previous studies on hCG binding); twenty-four hours later, half of the mice in each group were injected with

saline and the other half with 0.3 IU hCG/g bwt. Two hours after the second injection, the animals were bled and plasma testosterone was measured by RIA. The results show that this dose of hCG is unable to produce any further stimulation of steroidogenesis in either type of mouse pretreated with this hormone at doses which cause a significant depletion of testicular hCG binding sites. Also, these results reinforce previous findings in that the deficiency of PRL, GH & TSH reduced the steroidogenic capacities of the testis. Similar studies in the golden hamster will be discussed.

1st Dose (IU hCG/g bwt)	2nd Dose	Normal Mice Testosterone	Dwarf Mice (ng/ml)
0	0	6.4 ± 3.1	1.8 ± 0.4
	0.3	57.3 ± 4.5	10.4 ± 3.1
0.1	0	54.4 ± 6.2	20.7 ± 6.2
	0.3	63.3 ± 9.1	19.2 ± 6.4
0.3	0	59.8 ± 8.0	26.8 ± 8.7
	0.3	58.5 ± 11.6	32.4 ± 9.3
0.9	0	63.3 ± 10.1	25.9 ± 10.3
	0.3	46.2 ± 6.5	24.4 ± 9.6

74. STUDIES OF ANDROGEN DEPENDENT Nb RAT PROSTATE CARCINOMA MODEL. Joseph R. Drago. *Milton S. Hershey Medical Center, Penn State University, Hershey, Pennsylvania.*

The Noble prostate adenocarcinoma model has been chosen for study in our laboratory unit because it has both androgen sensitive and independent forms. Studies that have been conducted regarding the androgen sensitive form the subject of this presentation, including effect of early castration on tumor growth, as well as the effect of combination castration and chemotherapy. Note is made of the fact that castrating these animals leads to significant tumor volume reduction over a period of 30 to 60 days, ($P < 0.01$). The use of early chemotherapeutic treatment with cyclophosphamide, 60 mgs/kg every week for three weeks or combination chemotherapy of cisplatinum and cyclophosphamide, has lead to significant tumor volume reduction greater than that achieved used in castration alone as a therapeutic modality, ($P < 0.01$). This model has characteristics that make it applicable to study prostate cancer tumor biology, especially in terms of regression of tumors with appropriate therapy, decreased incidence of metastasis with efficacious therapy and reduction in tumor volume.

75. CYTOTOXIC EFFECTS OF AMSA ON SPERMATOGENESIS IN MOUSE AND MAN. Miguel F. da Cunha, Marvin L. Meistrich, Mohamed M. Haq, and Marcia V. Finch. *The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.*

AMSA (4'-(9-acridinylamino) methanesulfon-m-anisidide) is an acridine derivative widely used in the treatment of leukemias and solid tumors, almost exclusively in association with other cytotoxic drugs. It is usually administered in several three-day courses at a dose of 40 mg/m²/day. We have studied the sperm production in a patient who received 12 courses of AMSA alone. Sperm counts and motility began to decline after the second course to reach total azoospermia at the sixth course. Treatment was interrupted temporarily after the ninth course. Pretreatment levels of sperm count and motility were recovered 13 weeks after the discontinuation of treatment, indicating no damage to testicular stem cells. We have attempted to duplicate these findings and establish a valid animal model for the study of cytotoxic damage to the testis, and eventually use animal data to make clinical predictions on drug dosage and schedule, and gonadal toxicity. C_3H mice were given single or fractionated doses of AMSA, ranging from 1.5 mg/m² up to the toxic level to the animals (100 mg/m²). Fractionated doses were administered in three consecutive days. Testicular sperm counts were performed 29 and 56 days after initiation of treatment. The 29th-day assay measures cytotoxicity to differentiated spermatogonia, and the 56th-day count reflects damage to stem cells. We found that doses above 33 mg/m² killed more than 90% of differentiated spermatogonia. Little or no effects on stem cells were observed at any dose. No appreciable difference in toxicity was detected between single and multiple injection regimens. Histological analysis of the testis is being performed to establish precisely the stages of the cells being killed. The results obtained in mice are in agreement with those observed in the treated patient, and with studies reporting low toxicity to other (eg, bone marrow) stem cell systems. (This investigation was supported in part by grant CA-17364, awarded by the National Cancer Institute).

76. EFFECT OF 8-BROMO-CYCLIC-AMP AND 8-BROMO-CYCLIC-GMP ON LH AND FSH SECRETION FROM THE SUPERFUSED MALE HAMSTER ANTERIOR PITUITARY GLAND. Wan-Song A. Wun, Albert S. Berkowitz, and James P. Preslock. *The University of Texas Medical School at Houston, Department of Reproductive Medicine and Biology, Houston, Texas.*

We have developed a continuous *in vitro* superfusion system for determining the secretion of LH and FSH from male hamster anterior pituitary glands (HAP). Superfusion of HAP with media (Medium-199 in Earle's salt) results in a stable release of LH and FSH for up to 10 hr, and LHRH stimulates a dose-dependent release of both LH and FSH. In earlier studies we demonstrated that the ophylline potentiated the LHRH-stimulated release of LH and FSH from superfused HAP. The following studies were therefore undertaken to determine whether cyclic nucleotides mediate the secretion of LH

and FSH from superfused HAP. In duplicate experiments, HAP were superfused with media alone, or with media containing 8-br-cAMP or 8-br-cGMP at concentrations of 10^{-3}M, 10^{-5}M, and 10^{-7}M. Superfusion of HAP for 3 hr with media alone resulted in a baseline release of approximately 600 ng/mg HAP/15 min for LH, and a baseline for FSH of approximately 80 ng/mg HAP/15 min. Superfusion with media containing 10^{-3}M 8-br-cAMP resulted in a 5-fold increase in LH secretion to a peak of 3200 ng/mg HAP/15 min, while FSH peaked at 300 ng/mg HAP/15 min (3-fold increase). Media containing 10^{-5}M 8-br-cAMP resulted in a 1.6-fold increase in LH secretion (800 ng/mg HAP/15 min) with no increase in FSH secretion, while 10^{-7}M 8-br-cAMP decreased the secretion of both LH (300 ng/mg HAP/15 min) and FSH (48 ng/mg HAP/15 min) from superfused HAP. Superfusion with the three concentrations of 8-br-cGMP all decreased the secretion of LH to 35% (10^{-3}M) and 60% (10^{-5}M, 10^{-7}M) of baseline secretion, while FSH secretion was ihibited to 70% of baseline secretion by all three concentrations of 8-br-cGMP. These results suggest that cyclic-AMP is involved in mediating the release of LH and FSH from superfused HAP, while cyclic-GMP is inhibitory to this release.

77. DEVELOPMENT OF A MODEL FOR CANINE BENIGN PROSTATIC HYPERPLASIA (BPH).

David R. Wade and Richard E. Falvo. *Southern Illinois University, School of Medicine, Carbondale, Illinois.*

Dihydrotestosterone (DHT) and estradiol (E), administered at pharmacological doses, in suitable combinations, cause BPH in dogs. In an attempt to cause BPH at more physiological steroid concentrations, three techniques have been used. Hormone concentrations were regulated by implanting steroid-filled Silastic capsules and were monitored by RIA. Subtle changes in prostatic size were continuously determined by suturing gold beads on the surface of the prostate and examining their distribution by x-ray (Wade et al, Invest Urol 1981; 18:266). In experiment 1, two castrated dogs were given periodic increases in DHT, each period being two weeks long. One dog also contained 2 E-filled capsules which significantly raised the plasma concentrations above intact levels. In the absence of E, eight DHT capsules were required to stimulate prostatic growth. However, only four DHT capsules were required to stimulate growth in the dog with E. Experiment 2 employed two intact dogs, both with T-filled capsules to ensure physiological T concentrations, and the second also having elevated E concentrations. Both dogs were then given increasing numbers of DHT capsules at two week intervals. Two DHT capsules induced prostatic growth in the dog which contained E capsules. In experiment 3, the effect of 5T, 2DHT and 2E capsules on prostatic growth of an intact dog was compared with an untreated control. The prostate of the treated dog grew with a doubling time of 86 days. The control dog had plasma steroid concentrations of T 1.39 ng/ml, DHT 0.36 ng/ml and E 3.8 pg/ml. The treated dog's values were T 1.24 ng/ml, DHT 0.42 ng/ml and E 22.9 pg/ml. These preliminary results suggest that at physiological plasma concentrations of T and DHT an increase in E can induce prostatic growth.

78. STUDIES OF THE DIRECT EFFECTS OF CERTAIN DRUGS OF ABUSE ON TESTOSTERONE PRODUCTION BY ISOLATED LEYDIG CELLS.

Pamela M. Scher, Ramona G. Almirez and Carol Grac Smith. *Uniformed Services University of the Health Sciences, Bethesda, Maryland.*

Studies in our laboratory have attempted to define the effects of certain drugs on the male reproductive system. 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 was designed to assess possible direct effects of ethanol (ETOH), Δ^9-tetahydrocannabinol (THC), morphine sulfate, phencyclidine (PCP), and ketamine on testicular production of testosterone. The *in vitro* studies consisted of an isolated Leydig cell preparation modified from the *in vitro* LH-bioassay of VanDamme, et al. (Acta Endocrinol 1974; 77:655). The Leydig cells were incubated for a 3-hour period in the presence of serum from ovariectomized Rhesus monkeys. The drugs were added to this serum. The final concentrations of the drugs were within the range of typical blood levels after single dose administration. Testosterone was measured by RIA. Comparisons were made between testosterone production by control and drug containing incubations. ETOH (200–2200 mg/dl), THC (100–1100 ng/ml), morphine (150–1650 ng/ml), and PCP (200–2200 ng/ml) had no significant effect on testosterone production. The presence of ketamine (200–2200 ng/ml) caused a significant reduction in testosterone formation by the Leydig cells (60–72% of control levels at 3 hours). These results demonstrate that *in vitro* production of testosterone is not altered by the presence in serum of pharmacological levels of ETOH, THC, morphine or PCP. Higher levels of these drugs or their metabolites, as well as the presence of ketamine, may cuse inhibitory effects on Leydig cell function and may complicate the use of the *in vitro* bioassay for LH. (Supported by NIDA grant RO1-2063 and USUHS).

79. DIRECT EFFECT OF BUSERELIN ON TESTICULAR ENZYME ACTIVITY.

A. Bélanger, Y. Tremblay, B. Marchetti and F. Labrie. *Laboratory of Molecular Endocrinology, Le Centre Hospitâlier de l'Université Laval, Québec, Canada.*

^{125}I-Buserelin ([D-Ser(TBU)^6, des-Gly-NH_2^{10}]LHRH ethylamide) binds to specific receptors in Leydig cells and administration of the peptide to hypophysectomized (HypoX) rats leads to loss of LH receptors, thus suggesting a direct action of the LHRH agonist in the

testis. We now report the direct action of Buserelin on the testicular steroidogenic pathway. HypoX animals were injected three times a day with 25 μg Buserelin, once a day with 5 IU hCG or a combination of both drugs for seven days. Sacrifice was performed 24 hr after the last administration of hCG. Treatment of HypoX animals with the agonistic analogue has no effect on the testicular content of pregnenolone (preg), progesterone (prog), androstenedione (Δ_4) and testosterone (T). Administration of hCG stimulates the 17-hydroxylase activity in isolated interstitial cells from 1.8 ± 0.01 to 4.5 ± 0.1 pmol/3 × 10^5 cells/h ($P < 0.01$) and the testicular steroid content of preg, prog, Δ_4 and T is increased from 2.9 ± 0.3, 0.2 ± 0.03, 0.5 ± 0.09 and 4.2 ± 0.5 to 10.4 ± 1.9, 4.0 ± 0.5, 45 ± 0.9 and 303 ± 41 ng/g testis, respectively. When the agonistic analogue is administered concomitantly with hCG, the stimulatory effect of hCG on 17-hydroxylase activity is inhibited to 0.8 ± 0.05 pmol/3 × 10^5 cells/h and the testicular levels of preg (47 ± 8 ng/g testis) and prog (10 ± 2 ng/g testis) are increased while those of Δ_4 (32 ± 4 ng/g testis) and T (72 ± 12 ng/g testis) are markedly decreased. By contrast to effects in intact animals, testicular 5α-reductase activity is not affected by treatment with the agonistic analogue alone or in combination with hCG. Moreover, like Δ_4-androgens, testicular 3α-androstanediol levels are lowered by treatment with the peptide (from 95 ± 16 to 46 ± 8 ng/g testis). These data suggest that the direct action of Buserelin on testis may well play an important role in the inhibition of 17-hydroxylase activity on Leydig cells while the testicular 5α-reductase seems under the control of different mechanism.

80. TESTOSTERONE METABOLISM BY CULTURES OF ISOLATED RAT INTERSTITIAL AND SERTOLI CELLS IN THE PRESENCE AND ABSENCE OF FSH. Robert K. Tcholakian, Michael J. Kessler and Anna Steinberger. *Department of Reproductive Medicine and Biology, University of Texas Medical School, Houston, Texas.*

Interstitial and Sertoli cells metabolize testosterone *in vitro* (Tcholakian and Steinberger, 1980). The testosterone metabolism and FSH effect on this process was investigated utilizing a novel approach for the isolation and evaluation of the substrate-derived metabolites. Three-day cultures of isolated interstitial and Sertoli cells from 18-day-old Sprague Dawley rats were incubated at 32 C in chemically defined medium with 0.25 μCi (1,2,6,7,16,17-^{3}H)-testosterone (135 Ci/mmol) in the presence or absence of FSH (5 μg/ml-NIH-FSH-S10). The culture media were harvested and replenished with fresh media of the same composition at 3-day intervals for a total of four harvests. The media from each harvest were extracted to background and the extracts were reacted with estradiol-17β(E_2)-antibody by a modification of procedures commonly used in RIA determinations of E_2. The free and antibody-bound components were separated by dextran-coated charcoal and each component was extracted to background. The steroid composition of the free and bound metabolites was investigated by high performance liquid chromatography (HPLC) in conjunction with a radial compression reverse phase (C-18) column system. Preliminary results indicate significant conversion of ^{3}H-testosterone to estradiol-like compounds by both interstitial and Sertoli cells. The HPLC patterns of antibody-bound steroids differ between interstitial and Sertoli cells, FSH treated and untreated cultures, and between the first and subsequent harvests of the culture media. The data further indicates that in both cell types, the isolated E_2-antibody-bound steroids consist of three distinctly different polar groups whose identity we are at present establishing by various chemical techniques. (Supported by NIH Grant PSO HD 08338).

Fertility Regulation: Poster Presentations (81–87)

81. CORRELATION BETWEEN SEMEN ANALYSIS RESULTS AND PHYSICAL EXAMINATION FINDINGS IN SUBFERTILE MEN. M. Perez-Pelaez, R. S. Jeyendran, and A. J. Sobrero. *Institute of Reproductive Medicine, Chicago, Illinois.*

The relationship between semen analysis results and the presence, or lack, of varicocele and/or leucocytosis in the prostato-vesicular fluid, as indicative of chronic or subclinical infection of the accessory sexual glands, was investigated. Male spouses of 253 consecutive infertile couples submitted 279 semen specimens for analysis. Sixty-three men showed analysis results within accepted normal limits and eleven men were azoospermic; both these groups were excluded from this study. Of the 179 men presenting abnormal semen analyses, 136 were available for physical examination. In 44 (32%) of these males a moderate to severe varicocele was diagnosed. In this group, 40 men (91%) presented an overall sperm motility of < 40% and 34 men (77%) showed > 60% morphologically abnormal sperm. Seventy-seven men (57%) examined had > 20 leucocytes (WBC) per high power field (HPF = 400×) in the prostato-vesicular fluid; these men also presented 6 WBC/HPF on the semen analysis. Of these men 60 (78%) showed an overall sperm motility of < 40% and 39 (51%) possessed > 60% morphologically abnormal sperm. Leucocytosis was cleared within three months in those men treated with broad spectrum antibiotics. The remaining 15 men (11%) were oligozoospermic but in seven sperm motility and morphology was graded as acceptable. When the different sperm variables studied were compared a striking difference was noted between the men with varicocele and those with leucocytosis in the ejaculate. A semen with poor sperm motility and morphology was indicative of varicocele, while the ones with poor sperm motility and 6 WBC/HPF indicated the existence of an infection of the accessory sexual glands.

82. ZINC THERAPY ALONG OR IN COMBINATION WITH VARICOCELECTOMY TO IMPROVE THE FERTILITY POTENTIAL OF THE MALE. H. Takahara, M. J. Cosentino, and A. T. K. Cockett. *University of Rochester, School of Medicine and Dentistry, Rochester, New York.*

Infertility in 101 men was treated for 60 days to two years with 440 mg zinc sulfate daily. Subjects exhibited low seminal zinc levels (< 15 mg/dl) and low seminal zinc and varicocele. Three separate semen evaluations were used to establish baseline values. Patients were divided into two groups. Group A was composed of 65 subjects who underwent zinc therapy alone. Group B was composed of 36 subjects who received zinc after varicocelectomy. In groups A and B, seminal zinc levels were significantly higher ($P < 0.02$ and $P < 0.01$ respectively) two months after the initiation of zinc therapy. There were no significant changes in sperm motility for patients in Group A. In Group B there was a tendency ($P < 0.07$) toward increased sperm motility at two months after zinc therapy, while after 12 months of therapy a significant ($P < 0.05$) increase in sperm motility was evident. We separated Group B into two subdivisions, with B1 patients being those that remained infertile despite treatment. Only those patients in Group B1 showed a significant ($P < 0.02$) increase in seminal zinc concentrations. These patients also showed a significant ($P < 0.05$) increase in sperm motility two months after initiation of zinc therapy. Patients in Group B2 showed a significant increase ($P < 0.02$) in sperm motility after 12 months of therapy. In Group A, 27.7% (18) of the patients successfully impregnated their wives, while in Group B, the success rate was 50% (18 patients). These data indicate that seminal plasma zinc concentration is an important factor in the regulation of fertility, although the exact mechanisms are unclear, and that zinc therapy is an effective treatment for infertile patients with low seminal zinc concentrations, especially following varicocelectomy.

83. EFFECTS OF SHORT- AND LONG-TERM VASECTOMY ON ANATOMIC, PHYSIOLOGIC, IMMUNOLOGIC, AND HEMATOLOGIC PARAMETERS OF THE RAT. Roman J. Miller and Gary J. Killian. *Kent State University, Department of Biological Sciences, Kent, Ohio.*

Male Sprague-Dawley rats were abdominally bilaterally vasectomized or sham-operated at 90 days of age and killed at 100 or 300 days later. Rats were put into four groups: 1. short-term vasectomized (ST-VAS), 2. short-term sham-operated (ST-SHA), 3. long-term vasectomized (LT-VAS), and 4. long-term sham-operated (LT-SHA), and evaluated for body weights, reproductive organ weights and volumes, protein concentrations and electrophoretic patterns of sera, sperm-agglutination titers, RBC and WBC counts, hematocrits, hemoglobin concentrations, and RBC indices including MCV, MCH, and MCHC. Means expressed are averages for all four groups. Body weights did not significantly differ due to operation type. Large granulomas were found in the vas deferens of vasectomized rats but not in shams. Granulomas in LT-VAS rats were almost 2× the size of those in ST-VAS rats. LT-VAS rats had testicular hypertrophy (1998 mg) and LT-SHA rats and testicular hypotrophy (1597 mg) when contrasted with the mean (1827 mg). The caput epididymides of LT-VAS rats weighed 25% more than those of ST-VAS rats, while the cauda epididymides in the same groups weighed 46% more. No sham rats had sperm-agglutination titers, but 43% of ST-VAS rats and 75% of LT-VAS rats had titers ranging from 1:16 to 1:64. LT-VAS rats had lower hematocrits (42%) and ST-VAS rats had higher hematocrits (49%) than the mean (46%). ST-VAS rats had elevated WBC counts (7217/mm^3) and LT-VAS rats had depressed values (4031/mm^3) contrasted to the mean (5465/mm^3). Absolute lymphocyte numbers were elevated in ST-VAS (6331/mm^3) and depressed in LT-VAS rats (3421/mm^3) from the mean (4776/mm^3). All vasectomized groups had increased numbers of large lymphocytes but decreased numbers of small lymphocytes compared to shams. The serum protein concentration in LT-VAS rats (4.5 g/dl) was lower than in LT-SHA rats (5.5 g/dl). These findings suggest that the trauma of vasectomy alters body homeostatic mechanisms in the rat.

84. COFFEE AND NICOTINE USAGE BY MALES OF INFERTILE COUPLES. Carol S. Sloan, Mary. G. Hammond, and Alma B. Vaughan. *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, North Carolina.*

Semen samples from 479 men attending an infertility clinic were examined for volume, sperm concentration, progressive motility, motile sperm density, and abnormal and dead percentages. Questionnaires completed by 399 gave information for both nicotine and coffee usage showing that over 82% were exposed to both nicotine and coffee. Preliminary evaluation showed 70 patients used no nicotine or coffee, 327 were current or previous smokers who drank coffee, and two patients smoked above 20 cigarettes and drank over eight cups of coffee per day. Non-users did not show significantly different mean values for the semen parameters from the moderate users with SAS analysis. Seven patients drinking above eight cups of coffee per day showed lower mean values for sperm concentration than non-coffee drinkers ($P = 0.27$). Nicotine users showed lower ejaculate volumes ($P = 0.08$) and sperm concentration ($P = 0.17$) than non-smokers. Exposure records should be kept to study interaction effects on semen parameters and fertility.

85. PHAGOCYTOSIS OF VASECTOMIZED RAT SPERM BY LEUKOCYTES. Y. Rikihisa, Y. C. Lin, and M. Dym. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia and School of Medicine, Georgetown University, Washington, D.C.*

In order to determine if sperm from vasectomized (VASEC) rats exhibit the features typical of normal sperm, studies of *in vitro* phagocytosis of sperm from normal and VASEC rats were undertaken. After 16 months of bilateral vasectomy or sham-operation as control, sperm were collected from the cauda epididymis of each rat. $1.0 \pm 0.1 \times 10^8$ and $0.5 \pm 0.1 \times 10^8$ sperm were obtained from control and VASEC rats, respectively. The sperm possessed a normal pattern of motility during the experiments. The guinea pig peritoneal polymorphonuclear leukocytes (PMNs) induced by casein-Na were incubated with sperm from VASEC and control rats in rubella medium for 0, 5, 15, or 30 min at 37 C and then fixed with 2% glutaraldehyde. The incubation mixtures were centrifuged at 150 g for 3 min at 4 C and the numbers of sperm remaining in the supernatant were counted. The phagocytosis of sperm ceased 15 min after incubation and the phagocytosis of sperm by PMNs in VASEC rats was two times higher than the controls. When the pellets were observed by phase contrast, scanning and transmission EM, 90% phagocytized control sperm appeared to be phagocytized by their heads. About 90% of phagocytized VASEC sperm appeared to be phagocytized by their middle piece. Increased phagocytosis of VASEC sperm by PMNs was not due to the presence of the autoimmune rat IgG on the surface of sperm, since immunofluorescent testing with a fluorescent labeled antibody against rat IgG was negative on the VASEC and control sperm. Ultrastructures of control and VASEC sperm were identical. Our results suggested that vasectomy appears to change rat epididymal sperm surface susceptibility to phagocytosis by PMNs, especially in their middle piece region.

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86. Abstract No. 86 has been withdrawn.

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87. EFFECT OF (+)-GOSSYPOL ON FERTILITY IN MALE HAMSTERS. Donald P. Waller, Nuntavan Bunyapraphatsara, and Harry H. S. Fong. *Department of Pharmacognosy and Pharmacology, University of Illinois at the Medical Center, Chicago, Illinois.*

The ability of gossypol, a disesquiterpene obtained from the cotton plant, to inhibit the fertility of several species of males has been demonstrated. The gossypol used in these experiments was probably the (±)-racemic mixture. However, the Chinese reported that the specific optical isomer (+)-gossypol did not effect the fertility of male rats. We have isolated (+)-gossypol from the bark of *Thespesia populnea* by extraction with petroleum ether and repeated recrystallizations. The antifertility effect of the (+)-gossypol was determined by mating proven male hamsters with estrous female hamsters and autopsy of the females on day 16 of gestation to determine if they were pregnant. Male animals were dosed for five weeks with 40 mg/kg of (+)-gossypol, 40 mg/kg of racemic (±)-gossypol or 5% gum acacia (vehicle control). All male animals were mated with two females. Both the vehicle control- and the (+)-gossypol treated-animals exhibited normal fertility. The racemic (±)-gossypol treated-animals were infertile. The data suggest that (+)-gossypol is not an effective antifertility agent in males and that the activity of racemic (±)-gossypol may be due only to the presence of (−)-gossypol.

Physiology: Poster Presentations (88–97)

88. DIFFERENTIATION OF HUMAN FETAL PROSTATE AND LEYDIG CELLS *IN VITRO*. Pirkko Kellokumpu-Lehtinen and Lauri J. Pelliniemi. *Departments of Radiotherapy and Anatomy, and Laboratory of Electron Microscopy, University of Turku, Turku, Finland.*

Androgens from the testis regulate both the normal and the pathological growth of the prostate. Knowledge of the regulation of normal development is required in order to understand the abnormal growth in hyperplasia and carcinoma of the prostate. Human prostate is different from that of animals, and it is therefore important to study human material. Organ culture of the Trowell type was chosen to compare the effects of testosterone and dihydrotestosterone *in vitro* and *in vivo* on the prenatal development of the prostate, and the effect of hCG in same conditions on the testis. Explants of human urogenital sinuses were cultured in the presence of androgens (10^{-7} mol/l) for six days. Androgens accelerated the differentiation of the secretory pathway organelles in the epithelial cells, but not before the first mesenchymal changes were evident *in vivo*. In addition the mesenchymal cells were better preserved ultrastructurally in hormone-treated explants and direct epithelio-mesenchymal cell contacts were seen more often, which suggests that mesenchyme regulates epithelial differentiation also in man. Gonads of the same embryos and fetuses were cultured with or without hCG (50 ug/ml). Leydig cells contained agranular endoplasmic reticulum from the seventh week. Gonadotropin-treated cultures contained more Leydig cells which supports the suggested stimulatory role of hCG in Leydig cell development. This type of organ culture and electron microscopic analysis allows us to

study small explants and to test different substances including carcinogens. It is likely that the method could be adapted for biopsies from the adult prostate for testing drugs and also for following the response to various treatments.

89. PROSTAGLANDINS AND CYCLIC NUCLEOTIDES IN THE RAM REPRODUCTIVE TRACT. M. J. Cosentino, N. E. Hastings, and L. C. Ellis. *Utah State University, Logan, Utah and University of Rochester, School of Medicine and Dentistry, Rochester, New York.*

The importance of prostaglandins (PG) in sperm transport is known and has been implicated in sperm maturation. It was the purpose of this study to examine various biochemical parameters pertinent to PG synthesis in sperm and fluids collected by cannulation, from the rete testis, cauda epididymis and vas deferens of conscious adult rams. In addition to the PGE and $PGF_{2\alpha}$ concentrations, the activities of the two enzymes of PG synthesis (phosphalipose A (PLA_2) and PG synthetase (PGSYN)) in the sperm/fluid samples from each of the sites described above were quantitated. We then determined the endogenous levels of cAMP and cGMP in addition to noting the effect of PGE on the sperm cAMP levels in these samples. Both PGE and $PGF_{2\alpha}$ concentrations were found to be significantly higher ($P < 0.01$) in the cauda epididymis and vas deferens than those of the rete testis, while only the PGE concentration exhibited a significant ($P < 0.02$) difference between the cauda epididymis and the vas deferens samples. The results of the enzyme studies show that both enzymes exhibited relatively high activity in the cauda epididymis and vas deferens sample when compared to that of the rete testis samples ($P < 0.001$). Therefore, the increase of PG concentrations in the more distal sample sites is probably due to the similar increases found in both the PLA_2 and PGSYN activities. Similarly, there was a concomitant rise in the sample concentration of both cyclic nucleotides ($P < 0.05$) as the sperm migrate through the epididymis. The results of incubating PGE_2 with sperm from various areas of the ram reproductive tract indicate that sperm cAMP levels increase in response to PGE_2 ($P < 0.05$) only after they have traversed the epididymis (ie reach the vas deferens). We conclude that since PG concentration in the ram reproductive tract increases as the sperm mature these compounds may be important to the maturation processm Furthermore, the influence PGs have on sperm is probably mediated by cAMP.

90. ESTROGEN SYNTHESIS BY IMMATURE RAT SERTOLI CELL *IN VITRO*. C. A. Suarez-Quian, M. Dym, A. Makris, J. Brumbaugh, K. J. Ryan, and J. A. Canick. *Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts.*

The cell site for aromatization in the testis has been the source of much controversy in the past; both the Leydig and Sertoli cells have been implicated in carrying out this function. We investigated the ability of Sertoli cells *in vitro*, isolated from the testes of immature rats, to synthesize estrogens in response to FSH. Sertoli cell cultures were prepared by a slight modification of the method of Dorrington and Fritz (Endocrinol 1975; 96:879) in that the enzyme sequence was reversed: collagenase treatment preceded trypsin digestion. The aromatase activity of Sertoli cells was examined by comparing simultaneously two different methods: 1. the conversion of 19-(6,7-^{3}H)hydroxyandrostenedione to (^{3}H)estrone and (^{3}H)estradiol using silica gel thin layer chromatography, and 2. the conversion of unlabeled 19-hydroxyandrostenedione to total estrogen (E1 + E2) using RIA. Our results indicated that FSH-stimulated Sertoli cells synthesized estrogens; the values obtained by the two methodologies employed were very similar. Further, the (^{3}H)estrogens made were authenticated as true estrone and estradiol by recrystallization to constant specific radioactivity. A dose response of FSH-stimulated aromatization, measured by RIA, was also obtained. Moreover, aromatase activity was examined in the presence of a cold estrogen trap. In this case, the amounts of (^{3}H)estrone and (^{3}H)estradiol produced were more than doubled suggesting that Sertoli cells further metabolized the estrogens produced. Although our results confirm that Sertoli cells synthesize estrogens, the quantities produced are small when compared with values reported in the literature for Leydig cells. In addition, the presence of aromatase activity in immature Sertoli cells should not be extrapolated to imply that the adult cell also possesses similar function.

91. DAILY MONITORING OF X-IRRADIATION EFFECTS ON THE TESTICULAR HISTOARCHITECTURE AND LACTATE DEHYDROGENASE OF A NON-SCROTAL BAT *RHINOPOMA KINNEARI* WROUGHTON. S. B. Lall and M. S. Singwi. *Department of Biologica Sciences, Kent State University and Department of Zoology, Udaipur University, India.*

X-ray induced (single dose:300 r) alterations in the testicular structure and lactate dehydrogenase (LDH) enzyme-isoenzyme system of a non-scrotal bat-*Rhinopoma kinneari* displayed significant but reversible changes. The observations refute the generalization that nonscrotal mammals are resistant to physico-chemical challenges. The aspermatogenesis induced by a low dose of x-ray is not only transient but reversible. Further, the changes occur within 24 hr of treatment. Histopathological changes were monitored daily up to seven days (D_0–D7). Regression of seminiferous tubule diameter, desquammation of germ cells, atrophy of leydig cells, hemorrhage, and thickening of lamina propria were some of the striking inductive changes. Pycnosis, cytolysis and chromatolysis were manifested by

germ cells and were used as signs of cytological aberrations and pathologies. Signs of "rebound" recovery occurred but slowly beginning at D_4. Histochemical site and distribution pattern of LDH of irradiated testicular cells differed markedly from those of sham-irradiated. Total LDH amounts of irradiated testes showed sustained lowering (D_1-D_4). Recovery began on D_5 and by D_7 reached a level approximately similar to control. Pronounced qualitative and quantitative changes occurred in the order of abundance and relative amounts of x-irradiated testicular LDH_1-LDH_5 and LDH_x, from D_1-D_4 as revealed by PAG electrophoresis vis-a-vis control. Recovery of isoenzyme profile begin on D_5 and attained nearly normal levels by D_7. The study also provides a good correlation between various changes that occur at structural and enzymological-isoenzymological levels.

92. MATING AND ADRENERGIC-STIMULATION INCREASE PROSTAGLANDIN BIOSYNTHESIS IN THE RAT SEMINAL VESICLE.

Robert E. Powers and Haldor T. Jonsson, Jr. *Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School, Boston, Massachusetts and Department of Biochemistry, Medical University of South Carolina, Charleston, South Carolina.*

Coital activity increased the concentration of prostaglandins (PGs) in the rat seminal vesicle (RSV). PGEs increased 1.7-fold as compared to unmated animals ($P < 0.05$), PGFs increased 1.9-fold ($P < 0.05$), and 6-keto-$PGF_{1\alpha}$ (6kF), a measure of prostacyclin synthesis, was increased 1.5-fold ($P < 0.02$), as measured by RIA. The ratio of PGE:PGF:6kF in the tissues was approximately 10:3:1. Epinephrine, at 3.75×10^{-7} and 3×10^{-6} M, increased both PGE and PGF levels in media from *in vitro* incubations at 5 minutes. This stimulation was antagonized by phentolamine (3×10^{-5} M), an α-adrenergic blocker. No stimulation of PG synthesis was observed to carbamylcholine or bradykinin at concentrations which stimulated contractions *in vitro*. We have previously reported direct spasmogenic effects of PGE_2, $PGF_{2\alpha}$, and prostacyclin in the RSV *in vitro* (Powers et al, 1981). In addition, others have noted enhancement of adrenergically- and cholinergically- induced contractions in the male accessory sexual tract (MAST) (Eliasson and Risley, 1966; Risley and Stahl, 1973). We propose the hypothesis that sympathetic input at seminal emission stimulates PG synthesis through α-adrenergic receptors, and these PGs contribute to the regulation of MAST motility during ejaculation.

Powers RE, Fredricks CM, and Jonsson HT, Jr. Endocrin Res Comm 1981; 8:45–48.
Eliasson R, and Risley PL. Acta Physiol Scand 1966; 67:253–254.
Risley PL and Stahl P. Biol Reprod 1973; 6:224–233.

93. EVIDENCE FOR UPHILL MOVEMENT OF ^{3}H-INOSITOL ACROSS THE EPITHELIUM OF THE RAT CAPUT EPIDIDYMIDIS.

B. T. Hinton and S. S. Howards. *Departments of Urology and Physiology, University of Virginia School of Medicine, Charlottesville, Virginia.*

Rat epididymal luminal fluid contains high concentrations of inositol which primarily originates from testicular and/or epididymal synthesis. There are limited studies showing inositol uptake into the epididymis; therefore, this study was undertaken to determine whether there is transport of inositol from 1. blood to tissue, 2. blood to lumen and 3. lumen to blood. Male rats were anesthetized, nephrectomized and injected with either 50 μCi or 1 mCi ^{3}H-inositol and at time intervals thereafter, the epididymis was either cut into regions (for group 1) or subjected to micropuncture for collection of caput luminal fluid (for group 2). In a third group of rats, the caput lumen was perfused with either 20 or 50 μCi ^{3}H-inositol. Tissue, luminal fluid and blood plasma were estimated for radioactivity. After 24 hr post injection of isotope, the tissue:blood plasma ratios (cpm mg^{-1} tissue ÷ cpm μl^{-1} blood plasma) for initial segment, proximal caput, distal caput, corpus, and cauda were 23.5, 12.9, 9.3, 11.3, and 2.6 respectively; lower ratios were observed after 1, 2 and 3 hr after injection. The caput luminal fluid:blood plasma ratio after 24 hr was 20.5. After 3 hr of luminal perfusion, less than 1% of total perfused radioactivity appeared in blood. The results suggest that ^{3}H-inositol is transported uphill (with a small outward movement) probably involving two transporting sites, one at plasma membrane, the other at luminal membrane. Furthermore, the transport capacity for inositol appears to vary along the epididymal duct, the highest transporting activity coinciding with the epididymal regions where sperm are developing motility and fertilizing ability. (Supported in part by The Rockefeller Foundation and NIH Grant HD14445).

94. AGE RELATED ALTERATIONS IN TESTICULAR STEROIDOGENESIS DURING SEXUAL DEVELOPMENT IN THE SUBHUMAN PRIMATE.

J. L. Pineda, B. C. Lee, B. Grekas, H. C. Sachs, T. J. Brown, and B. B. Bercu. *NPMB, NICHD, NIH, Bethesda, Maryland.*

During sexual development testicular steroidogenesis is modulated by gonadotropin secretion. Little is known about male gonadal maturation, and there are no systematic developmental studies. Macaca mulatta and fascicularis (n = 27) were studied from age 4 weeks through adult life. To define testicular maturation, hCG and GnRH stimulation tests were done. Under ketamine anesthesia, hCG (100 IU/kg) was given i.v. Blood was drawn for testosterone (T) and Δ^4 androstenedione (Δ^4) prior to and 60 minutes after hCG. On another day,

GnRH' (10 μg) was given i.v. and blood was drawn at −30, 0, 15, 30, 60, 120 min for T, LH and FSH. The data can be subdivided into the following developmental categories:

	n	basal T (ng/dl)	60 min T
postnatal (3–4 mo)	3	74 ± 11	457 ± 72
prepubertal (8–24 mo)	9	28 ± 5	51 ± 9
midpuberty (32–39 mo)	5	35 ± 2	165 ± 65

In late puberty (> 42 mo) the basal T levels varied and were slightly higher than in early puberty; however, their stimulated levels were increased. In adult animals (> 5 yr) basal T secretion ranged from 30 to > 850 ng/dl whereas stimulated T was increased. Δ^4 was as follows:

	n	basal Δ^4 (ng/dl)	60 min
postnatal (1–4 mo)	3	83 ± 9	177 ± 30
prepubertal (10–24 mo)	7	17 ± 2	20 ± 4
midpubertal (32–39 mo)	5	≤10	13 ± 3
late pubertal (45–47 mo)	3	≤10	31 ± 3
adult (>5 yr)	5	19 ± 4	70 ± 17

T response to GnRH stimulation correlated with hCG. Testicular volume and histological changes in light microscopy paralleled T secretion. In conclusion, the subhuman primate is a good model to study human sexual development. Because of the known pulsatility of T secretion, a single sample may not be useful. A short hCG stimulation test as presented here may substitute for static measurements in staging male sexual development.

95. CYTOPLASMIC ESTRADIOL RECEPTORS OF AGING RAT TESTES.

Grace C. C. Chen and Tu Lin. *Medical Service, Wm. Jennings Bryan Dorn Veterans' Hospital, and Department of Medicine, University of South Carolina School of Medicine, Columbia, South Carolina.*

Estrogen can inhibit testicular function both by inhibition of gonadotropin secretion and a direct effect on testicular 17α-hydroxylase, C17-20 lyase and 17β-hydroxysteroid dehydrogenase. The direct inhibitory effect of estrogen is probably mediated by binding to cytoplasmic receptors of Leydig cells. Plasma estradiol levels have been found to be elevated during aging. Accordingly, the present studies were performed to investigate whether impaired Leydig cell steroidogenesis in old rats might be associated with altered plasma estradiol levels and/or cytoplasmic estrogen receptor content. In each experiment, testes from one young (3–4 months) and one old (24 months) rat were collected and processed simultaneously. Cytosol fractions were obtained after homogenization of tissue in TRIS-EDTA buffer (pH 7.4) and centrifugation at 105,000xg for 60 min. Estradiol binding sites for the young rats averaged 5.6 ± 0.3 fmol/mg protein ($\bar{x}$ ± SE, n = 12), which was comparable to that of the old rats, 5.7 ± 0.3 fmol/mg protein (n = 12). Using Scatchard analysis, the association constants at equilibrium of estradiol receptor binding of the old and young rats were the same, $6.1 \times 10^{10} M^{-1}$. Plasma estradiol levels were also similar in both groups, 19.6 ± 2.8 pg/ml (n = 14) for the young and 19.2 ± 2.6 pg/ml (n = 10) for the old rats. Our results suggest that impaired testosterone biosynthesis in old rats was not due to elevated plasma estradiol levels or to differences in testicular estradiol cytosol receptor content.

96. SYNCHRONIZED SPERMATOGONIAL PROLIFERATION IN VITAMIN A REPLACED RATS.

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Spermatogenesis in vitamin A deficient (VAD) rats can be restored by vitamin A replacement. Although sperm produced in the post vitamin A replaced (PVA) are fertile, the quantitative normality of spermatogonial (Sg) proliferation has not been defined. VAD was induced in male rats by feeding them a VAD diet from weaning and subsequently supplemented with retinoic acid. At 120 days of age, VAD rats were fed 1 mg vitamin A and maintained on normal rat pellets. At various times PVA, testes were processed for whole-mounted seminiferous tubules (ST) or histology sections. At 40 days PVA, A1 Sg and preleptotene spermatocytes (PL) occurred in 41% of the 332 mm ST examined and A2 Sg in 35%. At 58 days PVA, intermediate (In) Sg were observed in 49% of the 174 mm ST and B Sg in 32%. At 61 days PVA, 48% of the 465 mm ST contained A1 Sg and PL and 35% contained B Sg. In contrast, Sg distribution in normal rats was: A1 Sg and PL:26%, A2 Sg:10%, A3 Sg:10%, A4 Sg:19%, In Sg:21% and B Sg:13%. It was observed in histological sections that there was a much higher frequency of the seminiferous epithelial cellular association containing specified Sg than is usually observed. At 61 days PVA, numbers of Sg and PL per 100 Sertoli cells were normal. The presence of a larger proportion of the tubules containing specific Sg suggests that Sg proliferation is synchronized in PVA rats. Since a much larger proportion of the examined ST is occupied by a particular cellular association, then a larger proportion of the whole testis at a particular time PVA may contain a single cellular association. Thus, testes of PVA rats may provide germ cell preparations of higher purity suitable for biochemical studies.

97. NALOXONE-PROVOKED LH RELEASE IN RAMS, WETHERS AND WETHERS IMPLANTED WITH TESTOSTERONE.

B. D.

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Seasonal changes in sensitivity of the hypothalamic-pituitary axis to gonadal steroid feedback are believed to be responsible for seasonal breeding activity in sheep. The neuroendocrine mechanisms responsible for these changes are not known but may involve opioid containing neuronal systems within the hypothalamus. Recent studies have demonstrated a functional role of endogenous opiates in the hypothalamus of rodents and man. To examine the possibility that endogenous opiates are involved in the regulation of LH secretion in male sheep, the opiate antagonist, naloxone, was infused in late spring into four rams, four wethers and four wethers implanted with testosterone-filled Silastic capsules. A 4-hour infusion of naloxone at 2 and 20 mg/hr had no consistant effect on the LH secretory profiles of rams, wethers or wethers implanted with testosterone. Naloxone infusion at 200 mg/hr, on the other hand, significantly altered the LH secretory profiles of these animals. Prior to naloxone infusion, rams were characterized by low serum concentrations of LH and no LH pulses were observed. All rams responded within minutes of naloxone infusion with a distinct LH discharge. Wethers and wethers implanted with testosterone were characterized by rhythmic LH pulses which became more frequent during naloxone infusion. In contrast to nonimplanted wethers, the increased frequency of LH pulses during naloxone infusion in testosterone-implanted wethers was accompanied by an overall increase in mean serum LH concentrations. These results suggest that endogenous opiates participate in the neuroendocrine mechanisms which regulate LH secretion in male sheep. Further studies are needed to investigate the possibility that opioid peptides participate in endocrine changes which impose seasonal breeding activity in this species.

Clinical Andrology—B: Poster Presentations (97–105)

98. TEACHING OF ANDROLOGY IN THE MEDICAL SCHOOLS OF THE USA AND CANADA.

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In the last decade, several new medical fields have emerged. These include andrology and, consequently, there is concern whether andrology receives adequate emphasis in the medical schools' curricula, at both the undergraduate and graduate levels. To evaluate the effort dedicated to the teaching of andrology, questionnaires were sent to the deans of 139 medical schools in the USA and Canada. To the present, 32% of the schools have responded to these questionnaires that sought information on the time dedicated to the teaching of male reproductive functions and pathology, at four levels: a. undergraduate, b. graduate, c. continuing education, and d. organization of the courses and departmental responsibilities in teaching andrology. The results obtained to date on the teaching of andrology at the undergraduate level are shown in the Table. The distribution of this effort and its administration will also be discussed. Another aspect of the inquiry was finding whether the medical schools had organized groups dedicated to the clinical and/or investigative aspects of andrology. The results show that 44% of the schools have at least one such group. Other results show that 20% of the schools subscribe to at least one of the four existing andrology journals. The conclusions that can be drawn are that with few exceptions, andrology occupies very little time in the medical schools' curricula at any level. Also, the impact of andrology-related publications on medical students, residents and physicians is, in general, very limited.

Hrs Dedicated to Andrology/4 years	% of Schools
1–10	33.5
11–20	4.4
21–30	11.1
31–40	4.4
> 40	2.2
unknown	44.4

99. RESULTS OF A SELF-LIMITED EXPERIENCE IN INSEMINATION AFTER SEPARATION OF X AND Y SPERMATOZOA FOR SEX PRESELECTION.

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Twelve couples who desired a male progeny were counselled and selected for homologous artificial insemination (AIH) after sex preselection. X and Y bearing spermatozoa were separated and fractions rich in Y sperm were isolated according to Ericsson's technique (Nature 2 1973; 46:421) from the 20% human serum albumin (HSA) isolation fraction. The *in-vitro* separation confirmed the results obtained previously with a 20 to 25% increase in Y spermatozoa. Six conceptions occurred and the ratio male to female offspring did not parallel the ratio of Y to X sperm in the final fraction used for insemination, since the results were four females and two males. After such preliminary results the remaining couples were advised not to pursue this experiment.

100. A PROBLEM-BASED APPROACH TO TEACHING THE MEDICAL SCIENCE BASIC TO UROLOGY.

David R. Wade, Robert H. Colvin and Richard E. Falvo. *Southern Illinois University, School of Medicine, Carbondale, Illinois.*

Most of the emphasis in medical school is placed on memorization, and problem-solving is somewhat ignored. In an effort to meet these concerns Barrows and Tamblyn (Springer, Problem-Based Learning, 1980) have developed techniques to analyze the problem-solving skills of physicians and to teach these processes to medical students. The class is divided into groups of six students, each with a tutor who need not be a content expert but should be familiar with the clinical problem-solving process. The tutorial group is presented with a clinical problem (in this case a young girl with long standing incontinence). The students discuss the problem, pool any information they may have and take "actions" (defined as questions, physical examinations, or laboratory investigations). The results of almost all possible actions are contained in a patient encounter booklet (copies available). The students begin to formulate learning issues, areas where the students or tutor feels that study is required. Many of these issues will have been anticipated by the faculty, and some guidance for study is provided. Following this study period the tutorial group reassembles and continues with the case, taking further actions and defining new learning issues as appropriate. Learning issues of this case include the anatomy of the urinary system, its embryological development, the normal voiding process, vesicoureteral reflex, the anatomical requirements for a competent uteterovesical junction, and the clinical tools available for analyzing problems. This teaching method ensures that students develop effective problem-solving strategies early in their careers, learning pathologies are identified, students become self-directed learners, and basic science is retained because of its clinical context.

101. INHIBITION OF TESTICULAR STEROIDOGENESIS BY CHRONIC ADMINISTRATION OF A POTENT LHRH AGONIST, HOE 766, TO PATIENTS WITH CANCER OF THE PROSTATE. Nacia Faure, André Lemay, Alain Bélanger, and Fernand Labrie. *Department of Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec, GIV 4G2 and Department of Reproductive Endocrinology, St-François d'Assise Hospital, Quebec, Canada.*

The findings of decreased testicular androgen biosynthesis and reduced testicular, seminal vesicle and prostate weight after treatment of adult male rats with LHRH and its agonists, suggested the potential application of similar treatment in cancer of the prostate. Sixteen patients with histological diagnosis of carcinoma of the prostate were treated with the LHRH agonist (D-Ser(TBU),^{6} des-Gly-NH^{10}) LHRH ethylamide, or HOE 766, for periods up to 33 weeks. HOE 766 was self-administered either intranasally at the dose of 200 μg (four patients) or 500 μg (four patients) twice a day or subcutaneously (s.c.) at the dose of 50 μg once daily (eight patients). After intranasal administration of the peptide, plasma testosterone (T) levels were 45.5 ± 14.9% ($P < 0.05$) and 71.4 ± 8.9% ($P < 0.01$) inhibited, while the s.c. injection led to a 83 ± 3.2% inhibition ($P < 0.01$). The response to s.c. administration was also more rapid and more constant. Not only basal steroid levels were markedly decreased, but measurements at frequent time intervals during 24 hr periods show marked and rapid inhibition of the response to each administration of the peptide. In the same subjects, basal LH levels were not significantly affected but the response to HOE-766 was 44.5 ± 12.7% inhibited ($P < 0.01$). From these data, it appears that chronic administration of potent LHRH agonists could be a safe and effective means of reducing serum androgens in patients with cancer of the prostate. The only side-effects were a decrease in potency in the six patients who were sexually active before therapy and hot flashes in eight patients after 6 to 8 weeks of therapy.

102. THE AGING LEYDIG CELL VI. RESPONSE OF TESTOSTERONE PRECURSORS TO GONADOTROPIN IN MEN. E. P. Murono, H. R. Nankin, T. Lin, and J. Osterman. *Medical Service, Wm. Jennings Bryan Dorn Veterans' Hospital, and the Departments of Medicine and Physiology, University of South Carolina School of Medicine, Columbia, South Carolina.*

The effects of a single i.m. injection of human chorionic gonadotropin on circulating testosterone precursor levels at 0, 1–6, 24, 48, and 72 hr were examined in normal young adults (mean age 34 years) and normal aged men (mean age 74 years). Basal 0830-0900 h concentrations of androstenedione and dehydroepiandrosterone were lower in aged men while progesterone levels were not significantly different from young men. A significant biphasic increase of circulating progesterone was observed in young men, characterized by an early peak at 2 hr (33% above basal) and a secondary peak at 24 hr (49% above basal). In old men there were no increases in circulating progesterone levels following human chorionic gonadotropin treatment during the early (1–6 hr) or late (24–72 hr) periods. There were no discernable increases in circulating dehydroepiandrosterone levels following human chorionic gonadotropin administration in both groups of men. Androstenedione levels in young men did not change during the first 6 hr following human chorionic gonadotropin but increased significantly at 48 and 72 hr, while in old men there was a small peak at 4 hr (which was not statistically significant) and a secondary significant rise at 48 and 72 hr. However, early and late stimulated absolute levels for androstenedione were lower in the aged population. Thus, there are differences in precursor concentrations in the basal state and in response to human chorionic gonadotropin in aged men.

103. SPERM STRUCTURE AND FUNCTION IN MORE THAN 70-YEAR-OLD HUMANS. B. Baccetti, T. Renieri, M. G. Selmi, and P.

Soldani. *Institute of Zoology, University of Siena, Siena, Italy.*

Aged men, more than 70 years old, able to produce spermatozoa by masturbation have been selected. Most of them had sons. Some of the ejaculates show a high number of spermatozoa (till 160 millions), others are numbers (ranging from 1 to 20 millions cc), others are close to azoospermy, independent of the age. In a few cases all spermatozoa are immotile; generally from 9 to 20% can move. These findings are not related to age or to sperm number. Immature stages were not present in the ejaculate, spermatozoa were all mature, generally devoid of cytoplasmic droplets, thin and withered, with the classical shape. Acrosome were generally perfect, sometimes swollen, nucleus was generally well shaped, condensed with a normal protein content in the chromatin (very rich in S-S bonds). Mitochondrial helix and accessory fibers were normal. Axoneme was affected in about 50% of the examined ejaculates by severe alterations, as disordered arrangement, lack of microtubules or arms, with consequent immotility. In the motile fraction, recorded by cinematography, speed, wave amplitude and frequency are reduced. Testicular biopsies demonstrate a very low number of germinal cells, which nevertheless show a normal spermiogenesis and all the usual stages of sperm maturation. Sertoli cells are full of lipid droplets, and bear an unusually reduced number of junctions. The fertilization ability of the best donors has been directed *in vitro* using the zona free hamster egg system. No case of sperm penetration by aged donors has been observed, even if acrosome reaction normally occurred, and the egg surface was reached, while younger controls easily penetrated eggs. We believe aging results in poor fertilizing ability, due to the generally reduced mobility which prevents the penetration into the egg.

104. INDICATIONS OF DEFERENTOVESICULOGRAPHY (DVG) IN MALE INFERTILITY. Simon Marina. *CEA (Centro de Estudios Andrologicos), Valero, 7; Barcelona, Spain.*

Many authors refuse DVG in male infertility, because, they say, the DVG produces obstruction of the vas deferens. Since 1972, we have performed DVG in 226 patients with different andrological pathologies: hemospermia, infertility, anejaculation, etc. The technique employed was: a. dissection of the vas deferens near the epididymides tail, b. puncture of the vas deferens with n°-25 butterfly needle, and c. injection of hydrosoluble dye. By spermatic count before and after DVG we showed this technique does not produce obstruction of the vas deferens. We have correlated clinical history, semen analysis, and DVG pictures. At this moment, in our experience, the DVG is indicated in male infertility in a patient with hypospermia (< 1.5 ml); low seminal fructose level (< 150 mg%); acid pH; and high seminal citric acid level (> 700 mg%). Vas deferens were present in every patient. The spermatic count is not a useful parameter to replace DVG. In those patients only DVG permits the diagnosis of three different pathologies: giant utricle, obstruction at the ejaculatory ducts level, and obstructive vesicle deferential tuberculosis. The pathologies 1 and 2 may be cured by endoscopic surgery. In patients with unilateral seminal obstruction, the semen analysis does not give appropriate clues.

105. FURTHER DEFINITION OF THE IMMOTILE CILIA SYNDROME. Roger A. Williamson. *Department of Obstetrics and Gynecology, University Hospital, Iowa City, Iowa.* James K. Koehler. *Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington.*

A 30-year-old man was referred for infertility. He had also had chronic sinusitis, bronchitis and resultant bronchiectasis. A semen analysis on multiple occasions revealed a count of 60 to 80 mill/ml with normal morphology and 0% motility. ATP and ATPase did not induce motility. EM revealed either very short or absent dynein arms of the "A" microtubules associated with severe disorganization of the normal 9 + 2 configuration of doublets. In some sperm tails microtubules were totally absent. However, 93% of the sperm were alive as determined by supravital staining and capacitation occurred in normal fashion as judged by a recently developed staining method (Talbot and Chacon, Am J Prinatology 1981; 1:211). Moreover, in an *in-vitro* system using zona-free hamster ova, 6% of the ova showed penetration of the capacitated sperm. The implications of these findings will be discussed. Additional patients are being studied.