SLUGGISH RESPONSE TO ADRENOCORTICOTROPIN STIMULATION IN NEWBORNS WITH 21-HYDROXYLASE DEFICIENCY (CAH). G. Kalaitzoglou and M.I. New, Dept of Pediatrics, The New York Hospital-Cornell Medical Center, New York, NY 10021, USA

An unusually sluggish response to a single 0.25 mg dose of ACTH was noted in some newborns with congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21-OHD) diagnosed by molecular genetic studies. Four of the 11 (36%) affected newborns that we studied, demonstrated baseline and ACTH stimulated 17-hydroxyprogesterone (17-OHP) levels clearly higher than the unaffected newborns, but only in the range of values established for heterozygote rather than that for homozygote affected patients at an older age. Therefore, we set out to establish the range of adrenal steroid response of normal and affected infants in the newborn period. The serum concentrations of 17-OHP and Δ -4-Androstenedione (Δ -4-A), which have proven most useful in diagnosing CAH are summarized as follows:

	17-OHP (nmol/l)		Δ -4-A (nmol/l)	
	Baseline	Stimulated	Baseline	Stimulated
Affected (M=4; F=7)	Mean: 514.8 Range: 116.6-1075.4	Mean: 748.0 Range: 191.5-1721.4	Mean: 275.9 Range: 22.6-970.1	Mean: 231.3 Range: 94.1-432.1
Unaffected (M=6; F=7)	Mean: 1.8 Range: 0.0-4.0	Mean: 5.6 Range: 0.8-31.6	Mean: 1.1 Range: 0.0-2.9	Mean: 2.8 Range: 0.0-6.8

We also measured baseline and stimulated values of 17-hydroxypregnenolone (17-OHPreg), 11-deoxycortisol (DOC), cortisol (F), deoxycorticosterone (B), dehydroepiandrosterone (DHEA), testosterone (T) and the calculated ratios of 17-OHPreg/17-OHP, DHEA/ Δ -4-A and 17-OHP/DOC. In conclusion, the response of 17-OHP to ACTH stimulation in newborns affected with CAH may be lower than that of older infants and children but higher than unaffected individuals. Physicians should be alerted to the possible significance of only moderately elevated 17-OHP levels in the early neonatal period.

MULLERIAN INHIBITING SUBSTANCE EIA IS YIELDING NEW INSIGHTS INTO GONADAL PHYSIOLOGY AND PATHOLOGY. M.L. Baker and J.M. Hutson, Surgical Research Laboratory, Royal Children's Hospital Research Foundation, Melbourne, Australia.

Mullerian inhibiting substance (MIS) is a testicular hormone made by fetal and postnatal Sertoli cells, and causes Mullerian duct regression during male sexual differentiation. We established an EIA to measure MIS serum levels in a number of physiological and pathological conditions. Significant levels of serum MIS are found in normal males during the first year of life (1). MIS levels decline throughout childhood and are undetectable after pubertal development (2). MIS levels were examined in patients previously diagnosed with Hodgkin's Lymphoma. Nine adults (>16 years) had low but measurable levels of MIS. Patients with Hodgkin's Lymphoma have azospermia, suggesting a possible link between MIS secretion and the spermatogonia. In very low birth weight babies (~1500g), MIS levels in cord sera were significantly higher than in full-term controls (79.125 µg/L vs 36.8 µg/L; $P < 0.001$). MIS levels also were examined previously in infants and boys with cryptorchidism, where the levels during the first year were significantly lower than normal (3). This suggested a possible link between MIS and germ cell maturation, which is occurring at that time. Recently we have confirmed experimentally that MIS does stimulate germ cell maturation in the neonatal mouse testis *in vitro*.

- (1) Baker ML, Metcalfe SM & Hutson JM. J Clin Endocrinol Metab. 1990; 70: 11-15.
- (2) Baker ML & Hutson JM. J Clin Endocrinol Metab (In Press)
- (3) Yamanaka J, Baker ML, Metcalfe SM & Hutson JM. J Pediatr Surg. 1991; 26: 621-623

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HUMAN CHORIONIC GONADOTROPIN (hCG) FAILS TO STIMULATE GONOCYTE DIFFERENTIATION IN NEWBORN MOUSE TESTES IN ORGAN CULTURE.

Postnatally, the germ cell differentiate through several steps to form primary spermatocytes, which are required for postpubertal spermatogenesis. This development is postulated to be controlled by the hypothalamic-pituitary-gonadal axis. To test the effect of hCG on germ cell development, newborn mouse testes (n=75) were cultured *in vitro* for 7 days. Exogenous hCG (0.102.0 IU/ml) or Mullerian inhibiting substance (0.5 µg/ml) (MIS) were added to serum-free medium containing transferring insulin and retinoic acid or fetal calf serum. Normal germ cell development was seen with added 10% fetal calf serum ($P < 0.001$) or exogenous MIS ($P < 0.001$), but was absent with medium + growth factors or hCG. These results demonstrate that transformation of gonocytes to type-A spermatogonia and other differentiated germ cells is regulated by MIS rather than hCG. As transformation of gonocytes to type-A spermatogonia is deficient in boys with cryptorchidism, MIS may have a role in the clinical management of this common cause of infertility.

IMPROVED DETECTION OF POINT MUTATIONS IN THE HUMAN ANDROGEN RECEPTOR GENE BY DENATURING GRADIENT GEL ELECTROPHORESIS OF DNA HETERODUPLEXES UNDER STRINGENT DENATURING CONDITIONS. P. Ghirri and T.R. Brown, Department of Pediatrics, University of Pisa, Pisa, Italy, and Dept. Population Dynamics, Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, MD 21205, USA

Single nucleotide missense mutations in the human androgen receptor (AR) gene represent the underlying molecular lesion in most cases of complete or partial androgen insensitivity (AIS). Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified DNA fragments containing a 5' 40-base pair GC-clamp has been used successfully to detect point mutations in several human genes, including the AR gene in our laboratory (Mol. Endo 4:1759 (1990)). The sensitivity for detection of mutations can be further enhanced by the formation of DNA heteroduplexes and by narrowly defining the denaturing gradient formed by formamide and urea (i.e. 100% denaturant = 7M urea/40% formamide) specific for each region of the gene to be analyzed. In the present study, genomic DNA of normal subjects and those with AIS was amplified by PCR using primers specific for each of the eight exons encoding the human AR. Amplified DNA from affected subjects was mixed with that from a normal subject, heat denatured at 95 °C for 5 min and then allowed to reanneal at room temperature to form both homo- and hetero-duplexes. The DNA samples were electrophoresed at 60 °C on a 6.5% polyacrylamide gel containing a gradient of increasing concentrations of formamide and urea. The presence of ethidium bromide stained DNA species with differing mobilities within a single sample was indicative of heteroduplex formation and the presence of a point mutation which was subsequently confirmed by nucleotide sequence analysis. We detected three new point mutations in the AR gene. In one subject with partial AIS, amino acid residue 913 encoded within exon 8 is mutated [COC(Pro)→TCC(Ser); P913S]. In another subject with the partial form of AIS, codon 840 in exon 7 is mutated [CCT(Arg)→CAT(His); R840H]. In one subject with complete AIS, codon 732 in exon 5 is mutated [GAC(Asp)→TAC(Tyr); D732Y]. The use of a 35%-70% rather than the usual 20-80% denaturant gradient was required to detect the mutation in exon 8, whereas the heteroduplex analysis was critical for the detection of the mutation in exon 5. These findings demonstrate the utility of heteroduplex formation and stringent denaturant conditions to improve the detection of some mutations in the AR gene using DGGE. (Supported in part by NIDDK R01-43417 and CHR A190-01384-04)

RECEPTOR ACCESSORY FACTOR (RAF) ENHANCES SPECIFIC DNA BINDING OF ANDROGEN AND GLUCOCORTICOID RECEPTORS. S. R. Kupfer, K. B. Marschke, E. M. Wilson, and F. S. French, Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, NC 27599, USA

The biological responses to steroid hormones are mediated by a family of ligand-inducible intracellular receptors that activate or repress transcription of target genes. Protein-protein interactions are common among other transcriptional activators and may have important consequences for gene regulation by steroid hormone receptors. Using the mobility shift assay we have identified a factor that enhances specific DNA binding of truncated rat androgen (AR) and glucocorticoid (GR) receptors by 25-fold and 6-fold, respectively, through the formation of heteromeric complexes. This factor, designated receptor accessory factor, or RAF, also potentiates DNA binding of full-length human GR. RAF is temperature and trypsin sensitive and is present in a variety of cultured mammalian cells. By gel filtration RAF has a predicted molecular mass of 130 kDa. RAF enhancement of AR-DNA binding requires androgen response element DNA. RAF appears to interact directly with AR because deoxycholate, which interferes with protein-protein but not protein-DNA interactions, prevents RAF-AR-DNA complex formation. Furthermore, RAF activity is recovered from an androgen response element DNA affinity column only in the presence of AR. Mutagenesis of truncated AR fragments indicates that a region in the NH₂-terminal domain is required for RAF to enhance AR-DNA binding. The interaction of RAF with AR and GR suggests that RAF might influence the ability of these nuclear receptors to activate transcription.

N⁶-MONOMETHYL-L-ARGININE DIRECTLY STIMULATES *IN VITRO* LEYDIG CELL TESTOSTERONE PRODUCTION. M. Watson, M. Poth, and G. Francis, Department of Pediatrics, Walter Reed Army Medical Center, Washington, DC 20307, and Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA.

Recent studies suggest nitric oxide (NO) may function as both an intercellular and intracellular signal (second messenger). Changes in NO generation are thought to affect neural and immunologic activity, vascular tone, platelet adhesion, and selected hormone production. Arginine analogues such as N⁶-monomethyl-L-arginine (L-NMMA) are thought to inhibit intracellular NO generation and have been used to study the effects of decreased NO in physiologic systems. A single *in vivo* study has suggested NO modulates testicular endocrine function but it is unclear if this was due to changes in vascular tone, neuronal activity, or Leydig cell steroidogenesis itself. The present study was performed to evaluate the *in vitro* effects of L-NMMA on basal and human chorionic gonadotropin (hCG)-stimulated production of adenosine 3':5'-cyclic monophosphate (cAMP) and T by Leydig cells. Rat Leydig cell-enriched cultures (1x10⁶ cells/culture) were incubated 4 hr with L-NMMA, after which hCG (0.1 U/ml) was added. Levels of T in the media were determined at 20 hr and shown here as ng/10⁶ cells±SEM. (* $p < 0.02$, ** $p < 0.001$)

CONTROL	L-NMMA (µmol/ml)		
	0.1	0.5	1.0
BASAL	1.9±0.03	2.9±0.09**	3.34±0.07**
hCG	7.7±0.6	10.0±0.5*	10.2±0.6*

L-NMMA had no effect on cAMP production (not shown). We conclude that L-NMMA increases T production by both basal and hCG-stimulated Leydig cells without increasing cAMP. This suggests that NO may be a tonic regulator of Leydig cell T production.