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BRANCH OVERVIEW AND SUMMARY

We are pleased to present the fifth triennial report of the Reproductive Sciences Branch (RSB) of the Center for Population Research (CPR), National Institute of Child Health and Human Development (NICHD), for consideration by the members of the National Advisory Child Health and Human Development (NACHHD) Council. The report before you constitutes a representative sampling of the activities of the RSB from 1994 through 1997. It is a testimony to the efforts of the thousands of investigators, their trainees, and their laboratory personnel who received support from the RSB and applied such support to exploring the mysteries of that most fascinating attribute of the human species known as reproduction. Such research has been encouraged, stimulated, promoted and/or coordinated in concert with the joint efforts of the NICHD staff and our extramural scientist colleagues in the hope that the knowledge gained will contribute substantially to the betterment of human reproductive health.

The mission of the Reproductive Sciences Branch is to encourage, enable and support scientific research aimed at expanding our knowledge of the processes underlying the success or failure of human reproduction. Included within this mission is research aimed at expanding our understanding of fertility-regulating mechanisms that may, on the one hand, lead to new approaches for either alleviating or curing infertility (or preventing or treating disorders that impair fertility) and, on the other hand, discover new directions for contraceptive development efforts. The mission of the RSB can be summarized concisely in the simple phrase on the outer cover of this report-"Supporting Research for Wanted Pregnancies." For the conduct of the RSB program mission to be effective, it is a requisite that there be a strong partnership effort between the principal investigators directing extramural research in the reproductive sciences, the grantee organization sponsoring the investigator, the NIH peer-review process and the extramural programs of the NICHD. The RSB has met and is continuing to meet the responsibility for its role in this partnership by striving to maintain a broadly based program for financially supporting, facilitating and/or coordinating extramural research and research training projects and programs in the major fields that comprise the reproductive sciences. By developing and implementing the new research, research training, and information dissemination initiatives described subsequently in this report, the Branch has employed research-driven decisions to dynamically assist the shaping of the efforts of the field by either supporting the development of newly emerging research directions or stimulating the stalled progress of slowed research areas deemed to be of high national priority.

In carrying out the programmatic responsibilities described above, the Branch has employed two guiding principles. Our first has been to promote optimal support for individual, investigator-initiated, peer-reviewed research projects such as those supported by the R01 mechanism grant. From such research arise the unique discoveries that will lead to the next generation of medically relevant research advances. Our second has been to promote activities that will accelerate the transfer of newly discovered knowledge to the clinical practices of the medical disciplines comprising the reproductive medicine field. To carry out the responsibilities noted above, the Branch has been organized functionally into three programs:

1) the Research Base Program of individual grants and cooperative agreements; 2) the Institutional Research Programs consisting of the research centers and program projects; and 3) the Career Development Program that administers individual and institutional training and career development project awards.

The present report is designed to provide both perspective and insight into the organization and activities of the RSB and its staff. We hope that the highlights of the activities and progress from the Branch portfolio that follow will convey not only a sense of the accomplishments of the last three years, but also the hopes for even more exciting developments in the next three years. The power of the new cell and molecular research technologies offers clear promise for accomplishments leading to human reproductive health applications considered unrealistic as recently as five years ago. It is anticipated that we will see in the near future the converging fields of the assisted reproductive technologies (ARTs) and molecular genetic based medicine yielding truly remarkable advances in protecting and maintaining human reproductive health and treating its dysfunctional states.

Finally, the professional staff of the Reproductive Sciences Branch wishes to acknowledge gratefully the assistance of the following Branch support staff in the preparation of this report: Ms. Lori Krasner, Ms. Gloria Lubin, Ms. Esther Lorenzetti, Ms. Arlette Cooper; and the assistance of Ms. Darlene Levenson and her staff in the preparation of the fiscal data used in this report.

SPECIAL PROGRAM INITIATIVES

National Cooperative Reproductive Medicine Network (RMN)

The RMN was established in 1990 to carry out large, multi-center clinical trials in the areas of male and female infertility and reproductive diseases and disorders. By testing hypotheses in large numbers of patients enrolled in common protocols, answers are provided more rapidly than by individual centers acting alone. The six clinical sites, "Reproductive Medicine Units" (RMUs), are the University of Pennsylvania, University of Rochester, Baylor College of Medicine, University of California at Davis, Brigham and Women's Hospital, and the University of Alabama. There are also two satellite sites, at Magee-Women's Hospital, Pittsburgh and Kaiser Permanente, Santa Clara. The Data Coordinating Center (DCC) is at Columbia University. Dr. Donna Vogel, NICHD, functions as a partner in the role of Research Coordinator. The Principal Investigators of the RMU's and DCC, the Research Coordinator, and an independent Chair form the Steering Committee that makes decisions by consensus, regarding topics to be studied, protocol design and execution, and report publication. A Data and Safety Monitoring Committee convened by the NICHD reviews clinical protocols and provides continued oversight of the study. The first completed protocol was a comparison of intrauterine vs intracervical insemination, each with or without gonadotropin stimulation of the ovaries. A second protocol, known as the "Fertile Male" study, is comparing semen analyses from male partners of infertile couples enrolled in the first study with fertile controls. Pilot studies for several new trials, on recurrent miscarriage, fertility-sparing treatment of ectopic pregnancy, and the utility of endometrial biopsy in infertility management, have recently been approved.

National Cooperative Program in Infertility Research (NCPIR)

As a consequence, of a number of major national advisory committee, panel and commission reports, legislative directives included in the 101st and 102nd U.S. Congress (U.S.C.) sessions stressed the need for NIH to enhance support for research on the diagnostic and therapeutic aspects of infertility alleviation. The NIH Reauthorization Act of 1993 issued from the 103rd U.S.C. and enacted into law on June 10, 1993, by signature of the President as Public Law (P.L.) 103-43 amended the Public Health Service (PHS) Act at Title X, Subtitle A, Section 1001 to authorize the Director, NICHD to make grants or contracts for the operation of two Centers to conduct activities for the purpose of improving methods for the diagnosis and treatment of infertility and to provide for the coordination of the efforts of such Centers. The RSB designed a new center mechanism, termed The National Cooperative Program for Infertility Research (NCPIR), to fulfill the requirements for a center program involving a national consortium of performing sites for this legislatively-directed research. This program completed its first phase in September 1996 with the centers having focused on translational preclinical and early stage clinical research designed to alleviate dysfunctional ovulation in women. Significant research results were published that ranged from advancing fundamental knowledge of the workings of the fertility controlling hypothalamic-pituitary-gonadal axis; to improved protocols for medically inducing ovulation in women experiencing infertility; the development of critical reagent principles

for diagnostic assays used in evaluating reproductive tract disorders, including cancer recurrences; and outcome costing research that had a major impact in identifying the high priority need to improve assisted reproduction technology (ART) efficiency in ovulation induction aimed at minimizing the high cost (personal and national medical economic burden) of multiple gestation pregnancies attendant to the present protocols for ART therapy for infertility. The recompetition of the NCPIR program in 1996 focused the attention of the NCPIR Centers as a consortium task group on the emerging potential of applications in genetic medicine useful in defining the genes underlying disease expression in polycystic ovarian syndrome (PCOS) and pharmaceutical developments for clinical therapy protocols aimed at both relieving the associated infertility and preventing the significantly elevated, long-term risks of PCOS women for cardiovascular diseases, diabetes, and cancer. The NICHD-NCPIR program presently has two National Center for Infertility Research (NCIR) sites at the University of Pennsylvania and the Massachusetts General Hospital affiliate of Harvard University which coordinate projects at the Brigham and Women's Hospital (Boston), Massachusetts General Hospital (Boston), University of Pennsylvania (Philadelphia), Pennsylvania State University (Hershey), and Northwestern University (Chicago). The NICHD Research Coordinator for NCPIR is Dr. Michael McClure.

National Cooperative Program on Nonhuman In Vitro Fertilization and Preimplantation Development (NCPIVF)

This cooperative agreement program was initiated in 1986 with the primary goal of improving the culture conditions for nonhuman oocyte and preimplantation embryo development, as a way to provide some guidance for the rapidly growing clinical practice of human in vitro fertilization. The Program at present includes four principal investigators (at the University of Wisconsin, the Jackson Laboratory, University of Missouri, and Texas A&M University). Dr. Richard Tasca, NICHD, is the Research Coordinator. The scientific expertise of the group is varied and work is conducted on several mammalian species. Technical and conceptual results from the Program have been widely accepted and utilized to improve culture conditions in basic research on the mouse and hamster, as well as applied research on farm animals, nonhuman primates and in human infertility clinics.

National Cooperative Program on Markers of Uterine Receptivity for Blastocyst Implantation (NCPMURBI)

An important unresolved problem in the efficiency of infertility therapy using advanced assisted reproductive technologies (ARTs) is the effectiveness of medical support for optimizing the "window" of maternal receptivity for implantation of the transferred embryo(s). The goals of this program are to identify marker molecules that indicate that the uterus is in, or going to be in, the receptive phase of uterine sensitivity for blastocyst implantation, to characterize how these molecules are related to the process of blastocyst implantation, and to develop non-invasive assay methods for the identified molecules. Currently, clinicians have no useful molecular assay methods to diagnose endometrial conditions. For certain types of patients with implantation failure, the identification of markers may be useful in diagnosis and treatment. Since the process of implantation varies from species to species, the Program focuses upon various aspects of

implantation in different animal species and nonpregnant human uteri and then extrapolates the results for their possible application to the human implantation process. The major markers currently under investigation are molecules related to the EGF family, prostaglandins, mucins, hormone receptors, and calcitonin. This collaborative Program was initiated in 1992. The current group has five principal investigators (at M.D. Anderson Cancer Center, University of Texas, University of Kansas Medical Center, University of Illinois at Chicago, the Population Council, University of North Carolina at Chapel Hill), an independent Chairperson, and Dr. Koji Yoshinaga, NICHD, as the Research Coordinator.

Specialized Cooperative Center Program in Reproduction Research (SCCPRR)

On July 1, 1970, the Population and Reproduction Grants Branch in the Center for Population Research (CPR), NICHD announced the availability of funds to support Population Research Centers in the biomedical and social-behavioral sciences. The intent of these Center Grants was to provide funds for research program development and technical service core facility support that would facilitate multidisciplinary approaches to population research related to birth spacing and population sizing. With the creation of the Reproductive Sciences Branch (RSB) as an independent branch in 1978, developmental and oversight responsibility for Center Grants dealing with biomedical research in the reproductive sciences became centralized in this Branch. The RSB Centers Program was restructured in 1989 to allow a nationally open competition for the available number of centers. The randomly spaced submissions, previously resulting in uneven numbers of submissions causing considerable fiscal planning difficulties, were placed on an annual submission in response to a Request for Applications (RFAs) announcement specifying a greater emphasis on translational preclinical-clinical research. Over a five-year phase of restructuring ending in 1993, the Centers were administratively moved (carefully) into annual clusters having similar numbers of centers that would be considered for an award in each year. The RFA-based open competition significantly improved the quality of the applications received. Commencing in 1993, a multi-year process, stimulated by the National Performance Review Program of the U.S. Government, was undertaken to restructure all of the RSB Center mechanisms into programs offering clear outcome measures suitable for compliance with the targeted implementation expectations and deadlines set by the Government Performance and Results Act (GPRA) of 1993. A new center grant mechanism was developed for this component of the RSB Centers Program that provided consistency with other GPRA compliant RSB Center mechanisms. The program-project styled, U54 award mechanism now used provides coordinated support for research projects and core services. It provides greater design flexibility for applicant needs, less restrictive administrative policies, greater supportive scientific assistance from NICHD professional staff, greater program relevancy to the NICHD program mission, and a clearer means for performance-based budgeting decisions by the NICHD. All three components of the RSB Centers Program now have a Research Coordinator who is an RSB professional staff member that works closely with Center investigators in facilitating research collaborations and interactions within and between Centers with the ultimate goal of improving human reproductive health through accelerated transfer of basic science findings into clinical practice. The Research Coordinator for the Specialized Cooperative Centers Program in Reproduction Research (SCCPRR) is Dr. Louis DePaolo. The restructured SCCPRR began with an RFA issued in 1996, with the first four awards being made

on April 1, 1998. Including five P50 centers which elected to convert to the U54 cooperative agreement mechanism, the total number of SCCPRR centers is currently nine. They are located at: the Population Council, Stanford University, University of North Carolina at Chapel Hill, the Mayo Foundation, University of Washington, Baylor College of Medicine, University of Virginia, University of Maryland, and the Johns Hopkins University. Coordinated, intercenter Research Focus Groups are being actively formed to increase collaborations, reagent and expertise sharing and interfacing with NIH intramural and corporate collaborators. The restructuring of this component of the RSB Centers program is scheduled for completion with the final RFA announcement in 1999.

Contraception and Infertility Research Loan Repayment Program (CIR-LRP)

The RSB has successfully developed the first NIH educational Loan Repayment Program (LRP) for the extramural reproductive science community. In concert with enactment of the NIH Reauthorization Act of 1993 (P.L. 103-43), the NICHD was authorized in 1994 under Section 487B of the above amendment to the PHS Act to conduct an LRP operation for the extramural reproductive science community served by the NICHD. Approval of the Office of Management and Budget was achieved and funding was obtained sufficient to intitiate the LRP as a pilot scale operation. In early 1997, NICHD publically announced the availability of a loan repayment program under the auspices of the NICHD Contraception and Infertility Research Loan Repayment Program (CIR-LRP). The CIR-LRP provides for the repayment of educational loan debt for highly qualified health professionals (including graduate students) who agree to commit to a period of obligated service of not less than two years conducting research with respect to contraception and/or infertility. Initially, eligibility for participation in the CIR-LRP was limited to individuals who would be employed/affiliated at the time of participation with the five Congressionally-mandated, NICHD-supported extramural sites identified as Cooperative Specialized Contraception or Infertility Research Centers. Eligible sites for participation have recently been expanded to additionally include the NICHD Intramural Program, the SCCPRR, clinical performance programs for the Reproductive Medicine Network, and the soon to be established Women's Reproductive Health Research Career Development Centers.

Reproductive Sciences of the Americas Network (RSANET)

In 1995, an international research and research training network, the Reproductive Sciences of the Americas Network (RSANET) was established as an international initiative of the RSB. The rationales for establishing the RSANET emerged from the highly successful experience encountered in the intensely mentored Reproductive Scientist Development Program (RSDP) sponsored by the RSB for the career development of physician-scientists engaging a career in academic reproductive medicine and the declining number of young investigators entering the international network of population based biomedical research documented in workshop proceedings of the Institute of Medicine in 1994 and 1995. The guidelines and objectives for the RSANET program were developed at a workshop convened in May 1995 in Mexico City, Mexico, which included senior research program advisors from the private sector and government research organizations and agencies representing Argentina, Brazil, Chile, Mexico, Canada and

the U.S. The workshop and developmental activities for this intitiative were supported by a competitive grant award to the RSB from the U.S. Department of State's North American Free Trade Agreement (NAFTA) initiative program and development funding from the NICHD. The workshop produced a report with an initial three year plan of action designed to foster collaborative basic and clinical research and research training, provide advanced training in key research concepts and technologies for Latin American investigators collaborating with U.S. or Canadian research sites, establish an Internet-based electronic bulletin board for the rapid dissemination of communications, conduct an Internet-based research fellowship award program to support investigator career development, make available a reagent exchange program, and selectively disseminate information on career opportunities and scientific meetings in targeted areas of emerging importance to reproductive health research. All of these goals have been achieved. The RSANET advisory group plans to meet to evaluate the progress and future needs of the RSANET program in Bahia, Brazil in May 1999 in conjunction with the 10th World Congress on Human Reproduction. The Burroughs Wellcome Fund-NICHD Frontiers in Reproduction Program (BWF-NICHD-FIR) and the RSANET will co-sponsor a molecular endocrinology workshop co-organized by the President of the Endocrine Society, U.S.A., Dr. Larry Jameson and 15 leading RSB-supported reproductive endocrinology scientists.

Fogarty International Center-NICHD International Training and Research in the Population and Reproductive Health (ITRPRH) Program

The RSB actively participates in the ITRPRH Program administered by the Fogarty International Center in cooperation with the NICHD. This program, which commenced on October 1, 1995, provides access to foreign applicants for fellowships for graduate and post-graduate training supported by three Fogarty International Center Training Grants (D43) awarded to domestic U.S. sites with major research center programs in the Reproductive Sciences (University of Oregon Health Sciences Center-Beaverton, University of North Carolina-Chapel Hill, University of Pennsylvania-Philadelphia and one in Contraceptive Development (University of Virginia-Charlottesville).

Frontiers in Reproduction (FIR): An International Research Training Course

Stimulated by the development of the RSANET and recommendations from leading experts in training program development in the reproductive sciences, the RSB assumed a leadership role in coordinating the efforts over two years of nearly 100 extramural scientists in developing the structure and funding for conducting an international research concept and technology applications training course in the reproductive sciences. A 10,000 sq. ft. laboratory, lecture rooms, and dormitory lodging needs were arranged at the world acclaimed Marine Biological Laboratory in Woods Hole, Massachusetts. State-of-the-Art equipment was available on loan from leading manufacturers. Funding assistance to conduct the course and provide scholarships was provided by the Burroughs Wellcome Fund, NICHD, United Nations Educational Scientific and Cultural Organization (UNESCO)-International Cell Research Organization, the U.S. Department of Agriculture, the U.S. Agency for International Development/CONRAD Program, the Society for Developmental Biology, the Indo-U.S. Contraceptive and Reproductive Health

Research Initiative Program, the Charles B. Metz Foundation scholarship program and unrestricted funds from private donors. The first six-week course conducted by leading international scientists commenced on May 26, 1998, and concluded on July 5, 1998. Two symposia, one domestic and one international, were held in conjunction with the course. The Class of 1998 consisted of young scholars with demonstrated promise who are practicing research investigators at the experienced post-doctoral training or early independent career phase of their careers. Of the applications received from 21 countries in targeted regions of the world, the 52 eligible for consideration were sent to an evaluation panel of senior science reproductive sciences experts who used an NIH format to score the merit of the applicants. The 16 class positions were filled according to the merit level received by these applicants. The course provides three segments of reproduction oriented cellular and molecular biology (2 modules), immunology (1 module) and endocrinology (2 modules). The modules range in coverage from imaging approaches to manipulating genes and germ lines to produce models for pathophysiology studies.

Indo-U.S. Contraception and Reproductive Health Research and Training Initiative

Recognizing the commitments made at the 1994 International Conference on Population and Development (Cairo, Egypt) and the 1995 Fourth World Conference on Women (Beijing, PRC), the Governments of India and the U.S. reviewed the scientific accomplishments of prior Indo-U.S. collaborative agreements in population research and commissioned the development of a new initiative program. The RSB participated in developing the operating principles and guidelines for the newly envisioned initiative at an Indo-U.S. planning meeting held in New Delhi, India on October 27, 1997. At a subsequent binational meeting held on November 28, 1997, development of a five-year program was authorized on behalf on the U.S. Government by Donna Shalala, Secretary, Department of Health and Human Services, and Y. K Alagh, Minister of State for Power and Science and Technology, the Republic of India. The RSB contributed to developing the collaborative program component for the reproductive sciences aspects of the program in a subsequent implementation meeting held on March 23-24, 1998. The RSB subsequently succeeded in implementing the first reviewed and approved activity of this program in April 1998 by establishing an exchange scholarship program to support funding Indian nationals invited by competitive selection to attend the annual Frontiers in Reproduction training course, co-sponsored by the BWF-NICHD FIR Program at the Marine Biological Laboratory in Woods Hole, Massachusetts. Two Indian nationals were competitively accepted and completed the 1998 course.

Reagent Distribution Program

In response to increasing demands for research reagents by the scientific community, the RSB initiated a Reagent Distribution Program in 1991. The first reagent designated for distribution was an Estrogen Receptor (ER-alpha) antibody kit with an antibody and its peptide antigen generously donated by Dr. Jack Gorski of the University of Wisconsin. These materials were packaged and distributed through the National Hormone and Pituitary Program (NHPP). A total of 457 ER antigen-antibody kits were distributed to 347 investigators in the U.S. and 21 other countries. In 1997, Dr. Elizabeth Wilson of the University of North Carolina donated antibody raised against

androgen receptor and its peptide antigen for distribution. In addition to these reagents, recombinant human follistatin (Whittier Institute) and recombinant human activin-A (Genentech) were made available to investigators under Material Transfer Agreements (MTAs) reached with Genentech, a major biotechnology firm, for distribution commencing in 1993 and 1996, respectively. Recently, recombinant human inhibin-A, produced by an RSB Center Network External Core Facility established by the RSB at our Northwestern University Center with Genentech MTA approval has been provided to the NHPP for distribution.

Women's Reproductive Health Research Career Development Centers (WRHR)

The NICHD and the Office of Research on Women's Health (ORWH) recently issued RFA HD-98-004 inviting institutional career award (K12) applications for Women's Reproductive Health Research Career Development Centers. These Centers will support research career development of obstetrician-gynecologists, to be known as Women's Reproductive Health Research (WRHR) Scholars, who have recently completed postgraduate clinical training, and who are commencing basic, translational and/or clinical research relevant to women's reproductive health. The Centers will promote the performance of this research and transfer of findings by creating a bridge between clinical training and research independence. Up to eight awards will be made, for a period of five years. Each Center will support a minimum of three WRHR Scholars per year. This Centers program will increase the number and skills of obstetrician-gynecologist investigators through a mentored research experience leading to an independent scientific career addressing women's reproductive health concerns.

RESEARCH HIGHLIGHTS

INTRODUCTION

The selected highlights of research are representative of hundreds of incremental advances in our knowledge of human and other animal reproduction that have been supported by the RSB between 1994 and 1998. Some of the advances are very basic research, some are technological, others are clinical. We expect that this juxtaposition will clarify how research funds are used to solve problems associated with human infertility, to discover novel or improved contraceptive mechanism leads, or to develop fertility preserving treatment for the sequelae of reproductive disorders.

The research advances are organized into three major sections that will be presented in the following order: 1) female reproduction; 2) male reproduction, and 3) developmental biology of reproduction. These section titles were chosen as the most clearly organized way to represent the research projects that are funded by the Branch.

FEMALE REPRODUCTION

The female reproductive system is a dynamic system characterized by cyclic fluctuations in the secretion of hormones from the brain, anterior pituitary gland, and ovary. The effects of these hormones, in turn, result in the development of follicles, oocyte growth, differentiation, and maturation into a fertilizable egg; the release of an egg through the process of ovulation; and the preparation of the reproductive tract for implantation of the embryo. Understanding the underlying mechanisms governing this finely-tuned sequence of hormone changes and their resultant effects using various molecular, cell and animal models is critical to furthering our understanding of the physiology, and pathophysiology of human reproduction.

The principal organ systems involved in female reproduction are the brain, pituitary gland, ovary, uterus, oviduct, and vagina. The first two organ systems generally comprise the neuroendocrine level of control while the latter three are commonly grouped together as the reproductive tract. Investigations into the actions and interactions of the neuroendocrine-ovarian-reproductive tract axis form the basis for much of the research on female reproduction supported by the RSB.

Neuroendocrinology of Reproduction

The process of ovulation, the rupture of one or more ovarian follicles with subsequent release of mature eggs, requires a dramatic increase, or surge, in circulating pulsatile concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland. The preovulatory surges of LH and FSH secretion from the pituitary are brought about by enhanced pulsatile secretions of gonadotropin-releasing hormone (GnRH) from the hypothalamus of the brain, as well as a heightened ability of the pituitary gland to respond to GnRH. Failure to release sufficient quantities of either GnRH or the gonadotropins at this time of the cycle results in a failure to ovulate. Therefore, it is essential to understand the neuroendocrine events that trigger these hormonal bursts critical for ovulation.

Regulation of GnRH: Substantial progress in deciphering the neural circuitry involved in the regulation of GnRH secretion has been made on several related fronts by NICHD-supported investigators. First, regulation of GnRH secretion appears to be governed to a significant extent by the balance of excitatory and inhibitory inputs to GnRH neurons provided by the amino acid neurotransmitters glutamate and gamma-aminobutyric acid (GABA), respectively. Interestingly, the excitatory signal, glutamate, can be converted to the inhibitory signal, GABA, by the enzyme glutamic acid decarboxylase. Thus, one mechanism whereby steroids such as progesterone stimulate GnRH secretion may be through inhibition of the levels or activity of this enzyme which would increase the glutamate/GABA ratio favoring excitation of GnRH release. On the other hand, naturally-occurring opioid peptides such as b-endorphin appear to inhibit preovulatory GnRH/LH release through inhibition of glutamate action.

A second area of investigation has focused on the role of the ubiquitous free-radical gas, nitric oxide (NO), in regulating GnRH release since neural NO mediates the stimulatory effects of glutamate on GnRH release. Interruption of NO production blocks preovulatory LH release and

ovulation in rats, whereas increased NO levels stimulate GnRH secretion. Furthermore, the stimulatory effects of NO may be mediated in part by neuropeptide Y, a peptide known to promote eating, that was previously shown by other RSB grantees to be intimately involved in regulating GnRH/LH secretion.

Another key feature of GnRH regulation is the well established importance of progesterone in maintaining ovulatory cyclicity through actions on the brain and pituitary gland. More incisive new studies show that knockout (KO) mice designed to lack progesterone receptors (PRKO) also lack the ability to ovulate. This failure appears to be attributed partly to an inability of these PRKO mice to mount a normal preovulatory GnRH/LH surge.

A fourth and rapidly emerging area is the relationship between nutrition, the brain and reproductive competence. This relationship is well known, but poorly understood. A recent key finding has been the discovery of leptin, a protein produced by white fat tissue that can profoundly suppress eating, particularly when given to obese animals who are also infertile. Of considerable interest were the findings that such anorexic effects of leptin are accompanied by restoration of fertility in these overweight animals. New studies have identified leptin receptor gene expression in the hypothalamus, and in particular, neurons that express the proopiomelanocortin (*POMC*) gene. This is particularly significant since peptide products of the *POMC* gene have been implicated in the regulation of both GnRH secretion and feeding behaviors.

Finally, our knowledge of the regulation of GnRH was used in the treatment of cranial tumor patients who have developed an acquired form of hypogonadism as a result of surgery and/or radiation treatment. As many as 70% of women in this group have menstrual irregularities. What was not known was whether the treatment impaired GnRH release or the ability of GnRH to stimulate LH/FSH. To this end, nine women with hypogonadism following cranial surgery and/or radiation were treated with intravenous GnRH as part of the NCPIR. Seven of the women ovulated and all four patients desiring conception became pregnant. These studies demonstrate that fertility can be restored in women with acquired forms of hypogonadism resulting from treatment for cranial tumors in which normal GnRH release is impaired.

Mechanism of GnRH Action: The effects of GnRH on LH and FSH secretion are mediated by membrane-associated receptors located on gonadotrope cells of the anterior pituitary gland. The initial phase of GnRH receptor activation following binding involves proteins called G proteins which are known to link receptor binding with intracellular signaling molecules. Interestingly, the gonadotrope vigorously responds to acute GnRH exposure, but fails to respond to continuous, prolonged exposures to GnRH becoming in effect "desensitized." Such desensitization results in a shutdown of the reproductive system. This phenomenon is widely used clinically for the treatment of conditions in which the cessation of sex steroid hormone secretion is desired (i.e., endometriosis, breast, and prostate cancer, etc.), even though the intracellular mechanisms that result in a desensitized gonadotrope are obscure. Recently, however, new cellular proteins have been discovered that diminish the responses of G-protein-coupled GnRH receptors. Thus, desensitization could involve the activation of these new proteins. Secondly, it was determined that certain G proteins involved in mediating the GnRH response become redistributed within the

cell following GnRH exposure, perhaps providing another means to limit the cellular response to GnRH.

Regulation of LH and FSH Secretion: The dynamics of ovarian follicle development and rupture are highly dependent on properly timed increases in LH and FSH secretion and ovarian responses to these gonadotropins. While GnRH is the primary hormone controlling the biosynthesis and secretion of both gonadotropins, increases in LH and FSH during the female reproductive cycle of mammalian species including humans are at times discordant. Considering the physiological importance of differential gonadotropin secretion to follicle development, several investigators have focused their research efforts on better defining the neuroendocrine mechanisms underlying differential LH and FSH secretion. One such study revealed that the number of GnRH receptors on the gonadotrope cell surface may dictate which gonadotropin is preferentially secreted with low receptor numbers favoring FSH production.

In the early 1990s, NICHD-supported investigators uncovered an intrapituitary system for regulation of FSH. This system involved locally produced activin (FSH stimulator) and follistatin (FSH inhibitor), two proteins which were originally isolated from ovarian follicular fluid. The importance of this system for FSH regulation is demonstrated by the finding that mice devoid of pituitary activin receptors have lower serum FSH levels, although serum LH levels were not affected. Recently, extensive investigations have been conducted to determine the interaction of this intrapituitary system with GnRH. These studies have revealed that slow frequency pulses of GnRH decrease follistatin production and increase FSH release, while GnRH pulses of higher frequency increase follistatin production and decrease FSH release. In addition, activin can stimulate transcription of the GnRH receptor gene providing yet another means by which the hypothalamic and intrapituitary control of FSH converge.

Ovary

Oogenesis: Oogenesis is the process by which immature oocytes undergo cytoplasmic and nuclear maturation to become mature haploid eggs. RSB supports a wide range of studies on this topic. These include attempts to improve oocyte culture conditions in order to be able to study in vitro genetic, hormonal and environmental influences upon the development of immature oocytes into mature eggs ready for fertilization; to study the process of meiosis and the origins of chromosomal abnormalities; to determine the mechanisms of meiotic arrest; to study the roles of growth factors in oogenesis; and to study the roles of maternal gene expression on oogenesis and on early embryo development.

It is estimated that as many as 50% of the eggs of a woman in the fifth decade of life are chromosomally abnormal. It is also estimated that 15-20% of all human pregnancies are chromosomally abnormal, owing to meiotic and early cleavage division errors. Together, such errors are a leading cause of birth defects as well as a leading cause of human infertility. In some of these cases, the origin of these errors has been tracked to the earliest phases of meiosis in females. Two investigators have independently shown that the majority of oocytes in human females over the age of 35 have defects in the second meiotic metaphase spindle formation and/or

chromosome alignment. The long exposure of the ageing oocyte to a variety of environmental stresses may cause defects in oocyte growth that lead to poor egg quality and chromosomal errors. Thus, it is important to gain a better understanding of the normal process of meiosis in human females and the causes of chromosomal errors.

A prominent feature of egg development is the accumulation of maternal messenger RNAs (mRNAs) in the egg cytoplasm that are important in egg maturation and early embryo development. Many of these are stored in a form that prevents translation into proteins until the appropriate time and in the proper place. Recent studies have shown that uninseminated human oocytes, like amphibian and mouse oocytes, have very high levels of *c-mos* mRNA and Mos, the protein, that must be precisely regulated in order to exert control over the meiotic cell cycles. Mos seems to be particularly involved in allowing the activation of oocytes from meiotic arrest and their progression to the second meiotic metaphase where they remain poised for fertilization. With future technology development, it may be possible to assay the cytoplasm of a human egg for *c-mos* expression, or the expression of other marker molecules, in order to determine fertilizability and developmental potential.

The dissolved oxygen content of human follicular fluid is another marker candidate that might be useful for the evaluation of oocyte quality. Correlative studies have recently been done on chromosome/spindle normality of human oocytes with matched samples of dissolved oxygen content, levels of vascular endothelial growth factor (VEGF) and perifollicular blood flow from the corresponding ovarian follicles. When follicular fluids had very low oxygen levels, the corresponding oocytes had a high frequency of abnormalities in chromosome organization that would lead to serious developmental defects if the egg became fertilized. VEGF measurements of follicular fluid and color pulsed Doppler ultrasound analysis of blood flow indicated that VEGF might be important in blood vessel formation in the follicles and in follicular oxygen levels.

When an animal reaches sexual maturity, there is a cyclical recruitment of immature oocytes that then develop into mature, fertilizable oocytes. Our ability to understand the factors, both local and systemic, that control oocyte development could be greatly improved by better success in the culture of oocytes. Recently, primordial oocytes from a newborn mouse ovary have been shown to be capable of growth, maturation, development, fertilization and early cleavage in culture. These oocytes also have the potential, after fertilization and transfer to a surrogate female, to develop into live offspring. One male offspring survived many months and was fertile, but then prematurely developed a variety of pathological conditions. This promising line of investigation could provide new leads into the requirements for oocyte development that yield healthy offspring with no long-term physical or behavioral defects. Continued improvement of these culture systems for mouse and nonhuman primates are extremely important to the assisted reproductive technologies in humans in which the development of a healthy mature egg in culture is a key ingredient.

In this regard, the oocyte culture technology could be used in studying human oocytes of infertility patients with polycystic ovarian syndrome (PCOS). These patients have a large number of small ovarian follicles that seem to have arrested their development early and have difficulty

ovulating. These follicles generally contain immature oocytes. Relative to other infertility patients, the embryos obtained from ovulatory PCOS after fertilization are of poor quality and the pregnancy rate is lower than average. Other studies have shown that optimization of the ovulation induction regimen can provide embryos of good quality and developmental potential. Interestingly, key hormone-type molecules are found in decreased quantities in the follicle fluid surrounding PCOS oocytes. The ability to place oocytes from these patients in culture such as that described above would enable investigators to explore in detail the potential of these oocytes to develop normally and to determine how they differ with different hormonal regimens.

Folliculogenesis Folliculogenesis is the formation of the follicle, or "nest," of cells that surrounds and supports each developing oocyte as it becomes a mature egg cell. The earliest follicle in its most primitive stage of development consists of a single layer of follicular granulosa cells surrounding the oocyte. As the follicle grows and matures, the granulosa cells divide, forming multiple layers of cells, that separate the oocyte from the follicle wall, and a second compartment of follicular cells known as thecal cells, becomes evident. Follicles in advanced stages of development also contain a cavity (or antrum) filled with fluid derived from plasma exudate, but containing secretory products made by the follicular cells. Attempts to understand the complex events surrounding the growth and differentiation of follicles from the immature, primordial follicle stage, to the mature, antral follicle stage remain a major objective of the RSB program on ovarian biology.

The development of follicles to the preovulatory stage is highly dependent on interactions between the various cellular components of the follicle. Work supported by NICHD has provided new insights into these interactions. For example, it has been shown that production of a novel growth factor called growth differentiation factor-9 (GDF-9) is oocyte-specific in the ovary. Importantly, female mice deficient in GDF-9 are infertile, and this infertility appears to be due to a block in follicular development beyond the immature follicle stage. As well, the oocyte itself shows limited ability to undergo meiosis. These data would indicate that GDF-9 is required for advanced stages of follicular development, raising the intriguing possibility that antagonist analogs of GDF-9 may be used to prevent ovulation.

Folliculogenesis, up to the preovulatory stage, cannot proceed in the absence of FSH. FSH stimulates follicular development by binding to specific receptors on the cell membranes of granulosa cells. A grantee has produced a four amino acid fragment of the native FSH molecule that has no FSH activity but binds to the FSH receptor and blocks ovulation by preventing the natural FSH molecule from binding to the receptor. Again, as with GDF-9 analogs, peptides that prohibit FSH binding to its receptor provide new avenues for development of novel contraceptives.

One of the most important actions of FSH in the follicle is to stimulate production of estrogen, which then amplifies the local actions of FSH to promote follicle development. Estrogen must bind to nuclear receptors in order to exert its effects. For many years, only one estrogen receptor, estrogen receptor (ERa) was thought to exist. Recently, however, investigators discovered another ER called ERb. Interestingly, the expression of ERb in the ovary is higher than ERa,

implying a critical role of ERb in mediating the ovarian actions of estrogen. Such studies will likely provide valuable insights into the etiology of various ovarian disorders that compromise fertility.

While it is important to understand the processes responsible for the growth and differentiation of healthy follicles, it is equally important to understand the processes that cause a follicle to degenerate since most primordial follicles present at birth will never develop beyond the preantral follicle stage. Insights into the cellular and molecular underpinnings of follicular atresia have evolved from efforts to study the process of apoptosis or programmed cell death. The identification of apoptosis-regulating genes has aided our understanding of how apoptosis occurs in ovarian follicles, and the importance of this process in maintaining cyclic ovarian function. For example, ovaries of mice lacking a critical apoptosis-suppressing protein, bcl-2, were found to contain fewer primordial follicles and oocytes than ovaries from normal mice suggesting that bcl-2 may function to maintain a normal complement of germ cells and primordial follicles in the ovary. On the other hand, mice whose ovaries had been genetically engineered to overproduce bcl-2 showed a decreased incidence of follicular apoptosis and enhanced folliculogenesis. However, the ovaries of bcl-2 overexpressing mice also showed an increased susceptibility to ovarian germ cell tumorigenesis.

Ovulation: The process of follicular rupture is critical to the release of the egg into the female reproductive tract. In one study, it was shown that interleukin-1 (IL-1), a putative mediator of the ovulatory process induced by the LH surge, acts to increase the expression of a protein in follicular granulosa cells which is vital to increasing ovarian glucose uptake at the time of ovulation. In so doing, IL-1 may be acting to meet the increased metabolic demands imposed upon the follicle-enclosed oocyte at the time of ovulation.

It is well known that ovulatory dysfunction is a common perturbation in animal models of diabetes. An investigator has used an animal model to elucidate the mechanisms governing the normal ovulatory process and found that deficits in NO signaling within the ovarian microvasculature at the time of ovulation may compromise ovulation. The importance of NO appears to be in its ability to minimize the generation of harmful cellular oxidative products which may reduce vascularity of the preovulatory follicle.

Steroidogenesis: The process of sex steroid hormone biosynthesis is key to our understanding of reproductive function, since these steroids act at all levels of the reproductive axis. The rate-limiting step in steroidogenesis is the transfer of cholesterol from the outer to the inner mitochondrial membranes. It has been long recognized that this acutely regulated step requires a protein that is rapidly synthesized when steroid-producing cells such as in the ovary or adrenal gland are stimulated. After many years of effort, an investigator isolated and characterized this protein, and named it the StAR (Steroidogenic Acute Regulatory) protein. This fundamental discovery was immediately translated into a clinical application in that C-terminal mutations in StAR were found to be the cause of lipoid congenital hyperplasia, a potentially lethal condition in which the patient is unable to synthesize steroids of any kind. Clearly, the discovery of the StAR protein is a major accomplishment in the reproductive sciences that has and will continue to have

enormous impact on this and other research areas.

As noted in a previous section, the fertility-promoting effects of leptin have heretofore been attributed to its effects on specific brain neurons involved in the control of feeding and reproduction. Recently, leptin receptors have been found in the human ovary, prompting a grantee to examine possible direct effects of leptin on ovarian steroidogenesis by follicle cells. Using a well-established in vitro model system, an investigator found that leptin inhibited estrogen production, but had no effect on progesterone production. These data point to additional sites at which leptin can act to influence reproductive function.

Polycystic Ovarian Syndrome (PCOS): Exciting new advances in our understanding of the etiology of the hyperandrogenism of PCOS have recently been made with NICHD support. As androgens are the principal precursors for synthesis of estrogens, it was surprising that estrogen biosynthesis by follicles of women with PCOS was found to be low, relative to normal women. Recently, an investigator has found that ovaries of women with PCOS overproduce an inhibitor of the enzyme which converts androgens to estrogens. Along these lines, a separate study has found that PCOS women given the insulin-sensitizing agent, metformin, have a profound reduction in serum testosterone levels. Thus, new insights into the pathophysiology of hyperandrogenism in PCOS women offer hope for novel approaches to the treatment of this syndrome.

The increase in secretory bursts of LH from the pituitary gland seen in PCOS women prompted investigators to examine whether or not the ability of steroids to restrain LH secretion is compromised in PCOS women. These investigators found that higher serum concentrations of progesterone were necessary to lower LH secretion in PCOS patients relative to normal women. Since one of the actions of LH is to stimulate ovarian androgen production, these data suggest that perturbations in the neuroendocrine regulation of LH secretion in women with PCOS may precipitate or exacerbate the hyperandrogenic state in these women. In support of the former scenario, a grantee has generated mice which have been genetically modified to hypersecrete LH. These mice develop many of the sequelae observed in women presenting with PCOS including hyperandrogenism, anovulation and ovarian cyst development. Thus, this mouse may provide a valuable model to gain insights into the pathophysiology of the human PCOS condition.

Endometriosis: Endometriosis is a baffling disorder in which cells normally found in the uterine lining implant elsewhere in the body. It affects an estimated 10% of U.S. women of reproductive age, causes pain and infertility, and is responsible for 24% of the hysterectomies done in premenopausal women. Because the diagnosis currently must be made by surgery or laparoscopy, women with the disease, but without evident symptoms, are not diagnosed. Without this knowledge it is impossible to make an educated approach to risk factors, etiology, and strategies for fertility-sparing treatment. A new investigator has recently reported that a protein, tissue inhibitor of metalloproteinase-1 (TIMP-1), originally identified in endometrium and peritoneal fluid, can now be measured in blood. Importantly, levels were significantly lower in women with endometriosis than in fertile, disease-free controls, and levels of TIMP-1 increased after medical therapy. This protein could be a non-invasive marker for diagnosis, as well as for following the course of the disease during and after treatment. The availability of such a test would make

possible large studies of prevalence, be a tool for exploring how the disease develops, and increase the practicality of conservative treatment for both practitioners and patients.

A major challenge in diagnosing and treating endometriosis has been trying to understand how endometrial cells in unusual locations change into endometriosis lesions and cause symptoms. A promising lead is the finding that endometriosis patients have certain abnormalities of proteins called integrins. These molecules are involved in cell-cell adhesion, and may be related to markers of uterine receptivity, the ability to accept an attaching embryo. Interestingly, abnormal integrin patterns appear to be involved in both endometriosis and implantation failure. Recent studies using cultured human endometrium have shown that the ovarian steroid hormones, estrogen and progesterone, did not change the pattern of integrin expression. On the other hand, specific changes in several integrins, some increases, some decreases, were seen in response to other cellular messengers known as growth factors or cytokines. This may point to a new direction for understanding how endometriosis impairs fertility; and for treatment of endometriosis or prevention of early embryo loss.

MALE REPRODUCTION

The male reproductive system has two theoretically distinct, but interrelated, aspects: fertility and virilization, or maleness. While the general concepts of how the complex system works are well known, there are many unanswered questions. To briefly summarize, the processes of sex determination and embryonic development produce a male child, setting the stage for the virilization and onset of fertility that begin with puberty. As with female reproduction, the brain, specifically the hypothalamus, signals the pituitary gland to secrete LH and FSH in a pulsatile pattern characteristic of adulthood. LH and FSH have specific functions in the testis, the male gonad. FSH stimulates male germ cells to develop into mature sperm, a process called spermatogenesis. LH stimulates accessory cells, called Leydig cells, to produce sex steroids, especially testosterone, through a process called steroidogenesis. Male hormones are needed for optimal sperm production, as well as for sexual function, healthy blood and bones, and general well being.

Neuroendocrinology of Reproduction

Deficiencies of reproductive hormones can cause failure to undergo puberty and normal sexual development. When pituitary hormones LH and/or FSH are deficient, abnormal or inactive, the result is called "hypogonadotropic hypogonadism" (HH). In some cases, the abnormality is in the pituitary hormones themselves; in other cases, the abnormality involves GnRH, the hypothalamic hormone which normally stimulates release of LH and FSH. A number of these deficiency states (e.g. Kallmann syndrome and adrenal hypoplasia congenita) are hereditary, and in many instances there is a male predominance because the traits are X-linked. A physician investigator has identified an unusual type of hereditary HH in which GnRH is itself normal, but cannot act. The genes for pituitary GnRH receptors have mutations which interfere with production and function of the receptors: fewer receptors are present and they have an impaired ability to transmit the GnRH signal. Two different mutations were found in different parts of the gene, and the affected

individuals all had one copy of each mutation. Unlike the known X-linked syndromes, this type of HH has a different pattern of inheritance: it is "autosomal," or transmitted to both male and female children. Understanding the mechanism of this genetic disorder can open up new approaches to treatment.

In contrast to hormone deficiency diseases, purposeful suppression of hormones can be medically useful. An ideal hormonal contraceptive for men would prevent sperm production with no effect on libido, erection, or other androgen-mediated functions. One approach could be selective blocking of the action of FSH. Fragments of the LH and FSH receptors, called binding proteins (BPs), have been produced. These bind only their respective hormones, and block their action in laboratory assays; LH-BP binds LH but not FSH, FSH-BP binds FSH but not LH. To test whether FSH-BP could be used as a functional antagonist in vivo, a solution of FSH-BP was injected into immature male rats. This treatment decreased testis growth by 33% in two days. Further, analysis of DNA fragmentation indicated major increases in testis cell degeneration, or apoptosis. These results indicate that FSH-BP is capable of neutralizing the action of endogenous FSH, which is essential for testis germ cell survival.

Testis

Spermatogenesis: Sperm cells develop from spermatogonial stem cells in the testis. These stem cells reproduce by mitosis throughout almost the entire lifetime of human males. They can also enter a differentiation pathway leading to the formation of mature sperm. As they progress toward maturity, they undergo a series of remarkable changes to become spermatozoa. Their shape changes from round to elongate with a tail for propulsion, and their chromosome content is halved, during meiosis, to a single-copy or haploid state. Our knowledge of the process of spermatogenesis has been greatly hindered by our inability to culture spermatogonia and other sperm line cells in vitro, but some promising new advances may be on the horizon. Spermatogenic cells in the first step of meiosis have recently been cultured for 24-36 hours, long enough to observe some important chromosomal rearrangements.

In a dramatic development, it has been demonstrated for the first time that fresh or frozen spermatogonial stem cells can be transplanted from the testis of one mouse to the testis of another that has been either genetically or chemically depleted of sperm-forming cells. The transferred immature germ cells repopulate the recipient testis, develop into mature and functional sperm, and accord the host testis a normal structural appearance. This, then, establishes a test system for spermatogonial stem cells that have been removed, placed into culture, and subjected to a variety of treatments and analyses. Most importantly, this could represent a new method for producing and preserving lines of mice with virtually any desired genetic change. Another benefit would be the possibility of "banking" the spermatogonial stem cells of men about to undergo cancer chemotherapy. It might also be used to improve fertility for oligospermic men.

One of the changes that maturing germ cells undergo in differentiation is replacement of somatic-type histone proteins in the nucleus, first with germ-cell specific histones, then with different proteins called protamines. Between the germ-cell specific histones and the protamines is a brief

period in which the histones disappear as the chromosomes are unwinding. Then, newly described transitional proteins appear while the chromosomes are recondensing and the sperm head is elongating, prior to the appearance of the protamines. Next, the production of protamines is controlled in an unusual way that allows rapid adaptation to changing needs and conditions. For normal sperm function, the final step in protamine production must be delayed for up to eight days. An investigator has found that mutant mice with premature appearance of protamine had abnormal, infertile sperm. He has also found a protein involved in slowing down protamine production. This may explain the delay required for fertility in this system. This premature histone-to-protamine switch could be a model for some types of human infertility.

As sperm cells mature and acquire the ability to fertilize, they migrate from the outer rim of the seminiferous tubule to the center, where they enter the channel or lumen. We do not know how maturing sperm move along the Sertoli cells forming the tubule, to reach the lumen. Several converging lines of experimentation underway in a Reproduction Research Center are beginning to shed light on this problem. One of the features of the testis that enables developmental studies is the topography of the tubule. Maturing germ cells go through a series of stages in which certain developmental types are always found together. Cross sections showing these associated groups of stages allow mapping of features and markers to the steps in maturation. Recently, modern techniques have pinpointed a carefully coordinated and stage-specific collection of enzymes and other proteins that control the opening and closing of junctions between the cells. There are multiple steps that could be manipulated in interfering with development of fertilizing ability, without impairing sexual function, as a contraceptive lead.

A delicate balance of proper hormone levels is maintained through feedback signaling among the hypothalamus, pituitary, and gonads. Sex steroids are a critical part of this feedback control of reproductive hormone secretion, but not the only part. Other products are made by the gonads that are involved in communication with the hypothalamic-pituitary axis. In particular, a group of proteins called the inhibins exert feedback specifically on FSH, not LH. Studies supported by another Reproduction Research Center have been directed at the role of inhibin feedback in human health and disease. A new assay has made it possible to look at the forms of inhibin, A and B, that are composed of different subunits. This work shows that inhibin B is the physiologically important form in men. Serum levels of inhibin B were undetectable in men with no testes, and reduced in men with different reproductive disorders, namely gonadotropin deficiency, infertility with high FSH, and Klinefelter's syndrome (XXY). These disorders, although due to a variety of causes, have in common a loss or malfunction of the seminiferous tubules, the structures in which sperm cells are formed. Inhibin B may therefore prove to be a useful clinical marker for testicular dysfunction.

Steroidogenesis: Sometimes, potentially important regulators of testis function, can be discovered in other systems. Colony stimulating factor-1 (CSF-1) is a growth factor that regulates the survival, proliferation, motility and differentiation of cells of the mononuclear phagocytic leukocytes. A recent study, however, indicates that this growth factor plays essential roles in male reproductive function. In mice, when this gene is made non-functional by genetic manipulation, the males showed reduced mating ability, low sperm numbers, and 90% lower serum testosterone

levels. This low serum testosterone is due to reduced steroid production by testicular Leydig cells. There are also severe abnormalities in the microscopic appearance of the cells, such as disrupted intracellular membrane structures. In addition, the Leydig cells have diminished amounts of the steroidogenic enzymes necessary for testosterone production. These mice also have reduced serum LH and disruption of the normal testosterone negative feedback response of the hypothalamus. This unique role for CSF-1 will have a significant influence on our understanding of the classical endocrine feedback mechanisms and the involvement of immune cells in these processes.

Sperm Function: The introduction of new or changed DNA into animals (transgenic technology) has become an important and powerful tool for learning about gene function and the production of models of human diseases. The mouse has been the major animal used for transgenesis, but maintaining these special animals as adults or even as embryos is expensive and labor-intensive. It would be economical to keep an archive of transgenic material in cryopreserved (frozen) sperm cells, but mouse sperm have been notoriously difficult to freeze and thaw successfully. As part of an earlier RFA, encouraging work has now been done on a method to cryopreserve sperm from mouse strains commonly used to make transgenics. For four mouse strains tested, at least 50% of sperm were motile after freezing and thawing, and the cryopreserved sperm were able to fertilize mouse oocytes. However, with only one strain were the number of 2-celled embryos produced close to the number obtained with fresh semen. This is a promising beginning but clearly more improvement is needed.

***Human Male Infertility*:** An increasing number of gene defects are being discovered in the male germ line that are thought to account for a substantial amount of the roughly 40% of infertility that is male-based. Frequently, fruit flies have provided new leads into the genetic control of developmental programs such as the program that controls spermatogenesis. Four key fruit fly genes have been recently discovered that are required for normal progression of spermatogenic cells through the first meiotic cell division and for the onset of differentiation into mature sperm cells. Interestingly, mutations in these key genes lead to a pile-up of immature spermatogenic cells. This resembles a pathological situation in men in which spermatogenic cell development is arrested very early, also during the first meiotic division. The outcome of this first meiotic division arrest in humans is azoospermia, the complete absence of sperm from the semen. This adds to the increasing evidence for and interest in the genetic basis for human idiopathic male infertility.

A project supported by the 1994 RFA on Idiopathic Male Infertility has identified a specific mutation, a missing piece of DNA, in the Y chromosome of some infertile men who were azoospermic. The gene, named *AZF*, for azoospermia factor, with the same mutation has been found in a few men with extremely reduced numbers of sperm, and one of the men also had the mutation in sperm DNA. This is an important finding because it implies that the gene mutation responsible for infertility could be transmitted to offspring through assisted reproductive technology such as intracytoplasmic sperm injection (ICSI), discussed in more detail below.

Male Reproductive Tract

Male infertility is not infrequently characterized by a puzzling situation in which sperm are present but do not fertilize well. Although in rare instances, sperm can be removed from the reproductive tract and used in assisted reproduction to achieve fertilization, usually the sperm must undergo a process, as yet poorly understood, of maturation requiring passage through the epididymis, one of the accessory organs of the male tract. In this process, however, the sperm are exposed to damaging products of cell metabolism, and potentially to drugs and reproductive toxins as well. The epididymis has a system to protect the sperm from these insults. One of the major protective components is a substance known as reduced glutathione, which acts to prevent injury due to oxidation. RSB supported investigators studying a key enzyme in glutathione metabolism, gamma glutamyl transpeptidase, have found that, in the different segments of the epididymis, the synthesis and stability of the mRNAs is controlled in different ways by testosterone as well as other testicular products. This carefully orchestrated control of enzymes is required for protection of sperm from oxidation damage. Knowledge of its complex workings, both normal and abnormal, should shed light on certain types of male infertility, both spontaneous and exogenous, and may lead to prevention strategies.

For years, androgens were accepted as "male" hormones and estrogens as "female." Yet, both sexes normally have measurable levels of both hormones. In men, androgen deficiency has long been known to produce undervirilization. There is also a well-known syndrome of androgen insensitivity, in which target organs lack the receptor that allows the androgens to act. Until recently, there was no known role for estrogen in males. Then, in 1994, a case was reported of a man with estrogen insensitivity, in which estrogen could not act because of an estrogen receptor deficiency. A dramatic feature of this man was continuing growth of long bones. Researchers concluded that estrogen functions to mature and thus arrest the growth plate of long bones in both sexes. Around the same time, a mouse model of this disorder had been developed, in which the estrogen receptor was genetically mutated. In these Estrogen Receptor Knockout mice, the males are infertile. Interestingly, the testes of the mice are normal until puberty, but then they begin to shrink and produce few sperm. With a combination of mechanical and hormonal manipulations, the investigators were able to show that estrogen acts in the male reproductive tract to allow resorption of fluid from the ducts that connect the testis and epididymis. The back pressure of accumulated fluid is, at least in part, responsible for the testis damage in estrogen deficient mice. Both a and b estrogen receptors are present in the male reproductive tract, so even more functions of estrogen in male reproduction may be discovered.

DEVELOPMENTAL BIOLOGY OF REPRODUCTION

The developmental biology of reproduction encompasses those aspects of development that are most pertinent to reproduction. This includes fertilization, preimplantation embryo development, implantation, and sex determination. Oogenesis and spermatogenesis are clearly key processes in the developmental biology of reproduction, which have already been covered above.

Fertilization and Activation of Development

The interaction between egg and sperm that constitutes fertilization is a complex process of cell-cell interactions. Many investigators study sperm and eggs in order to determine which specific molecules may be used as potential targets for contraception. Often the goal is to interfere with sperm-egg interaction rather than to specifically destroy sperm or egg cells. Likewise, scientists want to understand the process of fertilization in order to determine how they may assist the process in infertile couples. Even though fertilization has been studied extensively for over a century, it has been less than four years since an RSB investigator discovered a molecule on the egg plasma membrane that is thought to be the egg receptor for sperm. Once a sperm successfully reaches the putative egg receptor, a set of cellular reactions are rapidly initiated that constitute the activation of development. Division of the fertilized egg into the 2-cell staged embryo is the first easily visible sign that development has begun.

Interestingly, the normal process of fertilization can be bypassed for the treatment of certain types of male infertility, using a new assisted reproductive technology called intracytoplasmic sperm injection (ICSI). ICSI is being widely used in infertility clinics to directly transfer whole sperm or sperm heads into mature human eggs that can then develop into live offspring after embryo transfer. However, this procedure has received serious criticism since it may pass on defective genes, as noted above, that would not normally be transmitted to the next generation. This is a striking case in which medical practice of a new technology moved ahead in the infertility clinics very quickly without the advantage of substantial prior animal studies, particularly with respect to long-term physiological, genetic, and behavioral outcomes.

Recent studies that bear upon this problem have been done by two laboratories using mouse models that carry defective genes in sperm that block normal fertilization. One investigator has recently shown that sperm that bear certain genes in the so-called *t*-region have very poor motility, bind ineffectively to the zona pellucida or outer shell of the egg, and cannot penetrate the cell membrane of the egg. Another investigator is studying the short and long-term effects of ICSI in strains of infertile mice carrying defective genes in the same genetic region as those mentioned above. Despite these genes causing three different aspects of dysfunctional fertilization, the use of ICSI allowed the mutant sperm to be just as successful as normal sperm in fertilizing eggs and in developing through the cleavage stages to make normal looking blastocysts. Future goals will be to study further development into live offspring, as well as long-term behavioral studies that should be of interest to ICSI clinicians and others.

Before a sperm can fuse with an egg, the sperm must undergo the acrosome reaction (AR), in which the acrosome, an intracellular membrane-bound sac, fuses with the sperm cell membrane and releases its contents to the outside of the sperm by a common secretory type of mechanism that requires calcium. In normal fertilization, the AR is caused by adhesion of the zona pellucida, the acellular outer protective coat of the egg, to the sperm. This adhesion apparently causes the entry of calcium into the sperm cell and this is thought to be the key initiator of the AR. It has been recently discovered that the zona pellucida induces the activity of a specific type of calcium channel, called the T-channel, that is used for calcium transport into the mature sperm cell. The

results of these experiments strongly suggest that only T-type channels are found in mouse sperm and directly connects T-channels with a key role in fertilization. These studies are particularly interesting since studies by others indicate that specific T-channel drugs have contraceptive actions in men.

Classical studies show that sperm induce a rise in the concentration of intracellular free calcium in the eggs of virtually all species studied as one of the very first detectable signs of fertilization. However, there have been few studies on the biochemical events that follow this rise in calcium and even fewer studies on the link between the biochemical events and the structural changes that then convert the egg into a single-celled zygote and subsequently into the embryo. An investigator has now identified a particular enzyme, protein kinase C (PKC), that seems to be activated by the high calcium concentration and then initiates a cascade of biochemical events that lead to the structural changes. This study especially points out that the process of fertilization and the activation of development are highly complex and that our level of understanding of them is still primitive, despite decades of experiments and observations.

Preimplantation Development

The preimplantation embryo is a central aspect of human reproduction since it spans from the zygote, the immediate product of fertilization, through cleavage divisions, into the blastocyst that implants into the uterus to establish pregnancy. As such, the preimplantation embryo is the principal element in the clinical practice of human in vitro fertilization and all of its various modifications, as well as in preimplantation genetic diagnosis, which is a pre-pregnancy alternative to amniocentesis and chorionic villus sampling for the detection of genetic diseases and disorders.

One of the most important needs in human in vitro fertilization and assisted reproductive technologies are clinically useful methods to enable the identification of embryos of the highest quality for embryo transfer in order to maximize the chances for the birth of healthy offspring and to minimize the need to transfer more than two embryos so as to avoid multiple births. An exhaustive study of hamster preimplantation embryo development in culture by time lapse videomicrography has been recently done as part of the NCPIVF. This study shows convincingly that embryos that develop faster to the 8-cell stage result in a two-fold improvement in the numbers of term fetuses obtained after embryo transfer. The rate of embryo development is one type of noninvasive parameter that is currently in use in human in vitro fertilization clinics to evaluate the quality of individual embryos.

Even with much success over the last 10 years, the culture media for preimplantation embryo development still need improvement in order to produce high quality embryos in vitro. A computer program developed by the NCPIVF has led to a series of progressively improved culture media of which the best to date, KSOM/AA (potassium simplex optimized medium with essential and non-essential amino acids), gives over 90 percent development of zygotes into blastocysts from various mouse strains tested. These media, in contrast with inferior media, have yielded mouse embryos with intracellular ion concentrations and rates of production of molecular markers, including specific mRNAs, that very closely match those measured in freshly collected

embryos. If these or other metabolic measures can be correlated with the development of live, healthy offspring, then they could serve as indicators of high quality preimplantation embryos.

Culture media developed first for mouse and hamster embryos have also been modified and used successfully for monkey, rabbit, and cow preimplantation embryo development. Indeed, the first case of cloning of monkey embryos was done with the assistance of the new media as part of the NCPIVF. These media have been used, with nonfederal funds, either directly or in principle, for human preimplantation embryo development in vitro.

Previous studies showed that glucose was not used as an energy source by very early cleavage stage mouse embryos and can be detrimental to embryo development to the blastocyst stage under certain conditions. Now, the effects of high glucose concentrations on mouse embryonic development in culture are being tested to try to mimic the effects that may occur in a diabetic animal. Levels of glucose and other compounds related to energy metabolism were greatly elevated in the embryos that developed most poorly in the hyperglycemic conditions. This may explain previous work that showed that drug-induced or genetic models of diabetic mice produce embryos that are severely delayed in their progression to the blastocyst stage either in vivo or in vitro. This developmental delay can be reversed by treating these mothers with insulin. These results are relevant to the situation in women who have poorly controlled insulin-dependent diabetes. These women are at increased risk of having spontaneous abortions or infants with major congenital malformations.

There is provocative new evidence from RFA-supported work that mouse and human eggs do exhibit cytoplasmic localization phenomena that were previously thought to exist only in lower vertebrates and invertebrates. Specific proteins found only in a certain location in eggs are distributed to only certain cells of the preimplantation embryo and may have an important role in blastocyst formation.

Implantation and Uterine Receptivity

While the blastocyst is being readied for implantation, biological decisions are being made about blastocyst hatching, the site(s) of implantation, signaling between the blastocyst and the maternal tissues and the initiation of tissue changes in the various layers of the uterus in preparation for implantation. Implantation is actually an elaborate sequence of carefully timed tissue-tissue interactions between the extraembryonic tissue (trophectoderm or trophoblast) of the embryo and the uterine tissues of the mother. One of the early interactions between the embryos and the uterus is thought to be through a hormone produced by human trophoblast cells called chorionic gonadotropin (CG). Appearance of CG receptors in the uterine luminal epithelium prior to implantation in primates suggests an important role of CG action on the uterus. Calcitonin and integrins are also considered to play important roles in implantation in humans and their mode of action is under investigation. One of the initial steps in implantation appears to involve removal of a barrier, a thick mucin coat, from the uterine surface. Muc-1 is a membrane-anchored mucin molecule which usually protects the epithelial cells from invasion of bacteria and embryo attachment. Removal of this mucin seems to be a prerequisite for the uterus to receive the

implanting embryo.

Some of the embryo-uterine interactions are strongly influenced by prostaglandins (PGs) that are produced by the uterus. Clues that PGs are important in implantation include their higher concentration at implantation sites, and the finding that inhibitors to PG synthesis interfere with implantation. Further, genetically mutant mice with the hormone-dependent enzyme for PG synthesis deleted, have been recently shown to have failure of implantation and decidualization of the endometrium. The action of PGs is mediated through receptors (PGRs). Two key PGRs can be found at a critical time during implantation and in appropriate uterine cells, suggesting that they are important to the implantation process. Specifically, the uterine contractions that are needed for embryo transport and positioning. Then, two subsets of PGRs appear in uterine epithelial and stromal tissues in the actual area(s) of implantation. These latter locations suggest that PGs affect the preparation of the uterus for the implanting blastocyst by causing local cellular and vascular changes.

Another critical element of implantation is the increased permeability of uterine blood vessels during and after implantation followed by the elaboration of new blood vessels (angiogenesis) in the vicinity of the implantation site(s). These phenomena are also carefully timed as part of the embryo-maternal interactions that are crucial for the attachment and invasion of blastocysts into the uterine lining during successful implantation. Recently, studies have been done on the production and action of vascular endothelial growth factor (VEGF) which is an angiogenic factor expressed in uteri and also is a potent inducer of vascular permeability. VEGF itself could only be detected in the peri-implantation stage uterus. Also, the timing and location of the expression of the genes for VEGF and its receptors in uterine tissue are quite consistent with key roles in implantation.

Sex Determination

An improved understanding of how sex is determined in humans is important since defects in this process lead to a wide range of abnormalities in human reproduction and sexuality, including infertility. Increased knowledge of sex determination can lead to more effective and safe ways to treat some of these specific disorders, such as pseudohermaphroditism. Progress in the last ten years has led scientists to the identification of specific molecules that may be of clinical use. In humans, sex is determined during fetal development by converting an indifferent gonad into either a male or female gonad. In the absence of a specific gene on the Y chromosome, a female fetus will form. In the presence of that gene, referred to as the testis-determining factor gene, the fetal tissues that would have become the female accessory tissues rapidly degenerate and the fetus becomes a male. Previous research revealed that the human sex-determining gene on the Y chromosome (SRY) is the testis-determining factor that is involved in sex determination that occurs during fetal development. The mechanism is thought to involve the SRY gene product directly interacting with the Mullerian Inhibiting Substance (MIS) gene in the embryonic testis. The MIS gene product in turn is the most important molecular marker of testis differentiation yet identified. If either of these two genes are not active, then a female embryo will be formed.

In the development of a male fetus, the testis-determining factor called SRY seems to play a partial role in development of a male by causing the onset of synthesis of the MIS by the Sertoli cells of the fetal testis. Later, other cells of the fetal testis called Leydig cells produce testosterone that then stimulates the development of the male reproductive system. It is becoming clear, however, that the SRY gene product, SRY, does not act directly upon the MIS gene. Recent evidence indicates that a steroidogenic factor (SF-1) does act directly upon the MIS gene and that this may be indirectly modulated by SRY. Also, there is an absolute requirement for a binding site for SF-1 in the regulatory region of the MIS gene. Gradually, scientists are starting to piece together the sequence of events that must occur in the course of normal development of males and females. Likewise, they are beginning to understand the causes of abnormalities in these processes that lead to defects in sex determination.

New reports indicate that even though the sexual determination process is highly complex in mutant mice, MIS itself is the only hormone-type molecule that reacts with the major MIS receptor. This is of substantial clinical importance since it indicates that, in mammals, the MIS signaling pathway for sex determination is relatively simple. In this regard, another investigator has cloned a human full length MIS type II receptor cDNA to add to the prior cloning of the same receptor gene in rodents. The availability of both type I and II receptors will be of much assistance in determining how these receptors interact in the MIS pathway. These are expected to be valuable tools in the design of novel therapeutic strategies.

POPULATION RESEARCH TRAINING AND CAREER DEVELOPMENT

Training, Fellowships, and Career Development Awards

The RSB supports the development of highly skilled reproductive sciences investigators through the following mechanisms: 1) individual postdoctoral fellowships; 2) institutional training grants; 3) career awards to funded scientists; and 4) career awards to clinicians.

Individual postdoctoral fellowships are awarded to newly trained young scientists, for up to three years of support, to enable them to work full time with a qualified mentor to develop expertise in an eligible field of reproductive sciences research. Between 42 and 52 individual fellows have been supported each year. Senior fellowships are designed to enable experienced scientists to make major changes in research direction, broaden their knowledge and capabilities, or allow them to devote more time to research through relief from other professional responsibilities. The Branch presently supports one or two senior fellows per year.

Institutional training grants are awarded to outstanding trainers at leading institutions in the United States to enable them to establish and maintain an appropriate environment for reproductive sciences research training of high quality. The number of institutional research training grants has been stable at 31-32, supporting 40-50 predoctoral and 70-90 postdoctoral trainees selected by the recipient institutions. In addition to the recipients of these institutional training awards, 32 predoctoral students were supported each year in two Professional Student Short-Term Research Grants. This latter program is designed to encourage talented students in

the health professional schools to choose research careers.

Research Career Development Awards (RCDA) were made to faculty candidates identified by their universities as having outstanding research potential. The purpose of this award was to release the selected candidate from teaching and administrative responsibilities for five years in order to devote full time to the development of a research program. During the reporting period, seven to nine RCDAs were active until this mechanism was replaced by the Independent Scientist Award (ISA) in 1995. ISAs active in RSB have risen to four, such that the number of this type of award has been stable.

Clinical Investigator Awards (CIA) and Physician Scientist Awards (PSA) were postdoctoral career development mechanisms to assist clinically trained individuals in developing their potential to become independent investigators. The CIA was a three-year and the PSA a five-year award, each enabling candidates to work with a respected mentor on a well-defined project in the reproductive sciences. The total number of active CIAs and PSAs has fallen from 17 to 8 as these mechanisms have been phased out. In 1993, NICHD replaced the CIA and PSA with a single mechanism, then in 1995, NIH streamlined the entire career award series, replacing these with the Mentored Clinical Scientist Development Award. Three to six of these awards have been active in RSB during the last three years. The RSB participates in a private sector career development program for obstetrician-gynecologists, the Reproductive Scientist Development Program. The program independently recruits and selects its own candidates. Participants undertake a Phase I research experience under a basic science mentor, with the second phase the initiation of an independent research program at the sponsoring institution. Nine to 12 young clinicians were directly supported by the program each year from 1994 to 1998.

The newest career development opportunity is the WRHR Centers, described earlier in the "Special Program Initiatives" section.

Equal Opportunity for Members of Underrepresented Minorities and Persons with Disabilities

Several tools are available to the program for increasing the participation of these underrepresented groups in research and research training. First, for training grants, there is firm policy that institutional training programs propose and carry out a plan for recruitment of minorities that is rigorously monitored for all new, competing, and noncompeting applications. Next, in the fellowship category, the F31 predoctoral fellowship, previously administered by NIGMS, has been re-issued as a program announcement from the Institutes and Centers generally. These competitive fellowships will be available to eligible candidates from minority groups or with disabilities. Finally, supplemental funds are available for support of minority high school, undergraduate, and graduate students, postdoctoral fellows, and new investigators. Seven supplements were made to RSB grants in 1994, five in 1995, seven in 1996, eight in 1997, and four in 1998 to date.

FUTURE PLANS

Clinical Reproductive Medicine Program

The RSB will continue priority attention to expanding the range of clinical trials to be conducted by the Reproductive Medicine Network. A wider scope of potential clinical trials of therapeutic approaches to treating fertility-impairing disorders of the reproductive tract will be considered by an extramural consultancy panel to be convened in 1999 and annually thereafter. Clinical research of the dimension carried out by this network has the potential to establish clinical practice guidelines having a significant medical economic impact at the national level. In this respect, it is expected that two new trials now in the approval/implementing stage and one in the planning stage will be initiated in a phased manner over the next 12 to 14 months. This should achieve the goal of annually maintaining three clinical trials in various phases of start-up to completion. Efforts will continue to expand both the individual and institutional research training opportunities for young physicians seeking to establish careers that include research in an academic medicine setting. This program will emphasize the further development of individual investigator conducted projects emphasizing areas of biomedical research of translational promise to clinical applications.

Preclinical Reproductive Sciences Program

The RSB will continue to actively promote both sustained levels of ongoing fundamental research support for the research science base and the development of initiative support for new programs addressing emerging priorities in reproductive cell and developmental biology, endocrinology and immunology research and research training. In addition, the Branch will seek to develop with priority, preclinical translational research activities designed to accelerate knowledge for a finer control of ovulation induction, gamete quality assurance, fertilization efficiency, embryo health improvement, enhancement of embryo implantation and advanced medical support of the early pregnancy. The growing rate of multiple births from medically assisted conceptions with the attendant higher risks of adverse consequences and the potential for adverse genetic consequences of microfertilization protocols presently representing the extremes of the ART field warrant increased animal model research to illuminate both efficiency and safety aspects. The emerging tools of genetic medicine will be brought to bear in initiative supported projects exploring the genetic basis underlying the pathophysiology of fertility impairing disorders of the reproductive tract, including dysfunctional hypothalamic-pituitary-gonadal axis inputs. Such research offers the longer term prospect of guiding the translation of the knowledge gained to improved clinical diagnostic and therapeutic procedures for preventing or alleviating fertility impairing disorders.

RSB PERSONNEL AND STAFF ACTIVITIES

RSB PROFESSIONAL STAFF

Michael E. McClure, Ph.D., has been with RSB since 1979 and has served as Branch Chief since 1989. He directs and coordinates the Branch effort to support a balanced, progressive

national program for research and research training in the reproductive sciences that will lead to new knowledge of human reproductive processes. In addition to his Branch Chief duties, he serves as the Research Coordinator for the National Cooperative Program for Infertilty Research and the RSB international career development programs. Dr. McClure received a masters degree in cell biology and doctorate in biochemistry with specialization in the molecular biology of regulated gene expression mechanisms. His research involved studies of nuclear and chromosomal protein-DNA interactions associated with regulating cell proliferation, steroid induced gene expression, and early embryo and adult somatic cell differentiation commitment mechanisms. He served on the academic faculty of the Cell Biology Department at Baylor College of Medicine and the Department of Developmental Therapeutics at the University of Texas System Cancer Center prior to joining the NICHD.

Louis V. DePaolo, Ph.D., joined the RSB in 1994 after completing the NIH Grants Associates Program. He is responsible for administering a portfolio of grants in reproductive biochemistry and molecular endocrinology. Dr. DePaolo also serves as the Research Coordinator for the Specialized Cooperative Centers Program in Reproduction Research as well as the Coordinator for the NICHD Contraception and Infertility Research Loan Repayment Program. He received his undergraduate degree in Zoology from Rutgers College, and his graduate degree in Physiology from the University of Maryland School of Medicine. Prior to coming to NIH, he was an Associate Professor in the Department of Physiology at the University of Texas Health Science Center in San Antonio and a Member in the Department of Molecular Endocrinology at the Whittier Institute in La Jolla. His research background is in the neuroendocrine control of female reproduction.

Julia Lobotsky, M.S., has been a member of the RSB since 1973. A graduate of Keuka College and the University of Rochester Graduate School of Medicine, she came to NICHD from the Worcester Foundation for Experimental Biology where she worked with Charles W. Lloyd in reproductive endocrinology in both the human and animals and taught in the Reproductive Biology Training Program. Ms. Lobotsky manages the program in Reproductive Biology and is the Program Liaison for the Data Safety and Monitoring Committee for the Reproductive Medicine Network.

Richard J. Tasca, Ph.D., joined the RSB in 1984, from the University of Delaware where he was Associate Professor of Biology and Associate Director of the School of Life and Health Sciences. He is a graduate of the University of Pennsylvania and Temple University. He manages programs in preimplantation development and the developmental biology of reproduction and serves as the Research Coordinator for the National Cooperative Program on Nonhuman In Vitro Fertilization and Preimplantation Development. His research background is in the genetics, molecular biology, and nutrition of preimplantation embryos.

Donna L. Vogel, M.D., Ph.D., came to the RSB in 1987, after having been in the NICHD intramural program since 1980. A graduate of Bryn Mawr College and the Medical Scientist Training Program at Albert Einstein College of Medicine, her clinical training is in Internal Medicine and Endocrinology, and her Ph.D. is in Developmental Biology. She manages the

Reproductive Medicine portfolio, a program of grants and cooperative agreements supporting research and research training relating to infertility and reproductive diseases and disorders of men and women. In addition, Dr. Vogel serves as the Research Coordinator for the Reproductive Medicine Network, the RSB Training Officer and a member of the Research Subcommittee, NIH Coordinating Committee on Research on Women's Health.

Koji Yoshinaga, Ph.D., joined the RSB in 1978. He is responsible for administering a portfolio of grants in reproductive endocrinology. In addition, he serves as the Research Coordinator for the National Cooperative Program on Markers of Uterine Receptivity for Blastocyst Implantation as well as the List Owner for the Reproductive Sciences of the Americas Network (RSANET). He received his B.S., M.S., and Ph.D. from the University of Tokyo and received postdoctoral training in the Training Program in Reproductive Physiology at the Worcester Foundation for Experimental Biology, and at the ARC Unit of Reproductive Physiology and Biochemistry in Cambridge, England. Dr. Yoshinaga was Associate Professor of Anatomy at Harvard Medical School before joining the RSB. His research background is in implantation and reproductive endocrinology.

PUBLICATIONS

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CONFERENCES AND WORKSHOPS

- Mammalian Developmental Mutants Workshop, May 10-11, 1994, Bethesda, MD
- Alternatives to Hysterectomy - Bench to Bedside, May 23-24, 1994, Bethesda, MD
- 23rd Annual Center Directors Meeting, Male Infertility and Impotence, August 2-5, 1994, University of Virginia, Charlottesville, VA
- Conference on The Germ Line, November 16-18, 1994, Bethesda, MD
- The Americas Reproductive Sciences Network Conference, May 5-6, 1995, Mexico City, Mexico
- Trans-NIH Conference on Endometriosis 2000, May 15-17, 1995, Bethesda, MD
- 24th Annual Center Directors Meeting, Conference on Reproductive Developmental Biology, University of California, San Francisco, July 14-15, 1995, San Francisco, CA
- Psychobiology of Infertility Workshop, September 21-22, 1995, Bethesda, MD
- International Symposium on Genetics and Outcomes of Assisted Reproductive Technology, Dutch-Speaking Brussels Free University Hospital, December 9, 1995, Brussels, Belgium
- Vaginal Immunology, April 9-10, 1996, Bethesda, MD
- Preimplantation Genetic Diagnosis: State-of-the-Art Workshop, April 25-26, 1996, Bethesda, MD
- Transgenic Mouse Sperm Cryopreservation, May 6-7, 1996, Bethesda, MD
- 25th Annual Center Directors Meeting,Conference on Molecular Biology, Baylor College of Medicine, May 20-21, 1996, Houston,TX
- Human Uterine Receptivity for Blastocyst Implantation, June 10-11, 1996, Bethesda, MD
- Androgen Receptors: Exploiting Differences in Hormone Action, July 23-24, 1996, Bethesda, MD
- Workshop on the Neuro-Immune-Endocrine Axis and Infertility, March 13-14, 1997, Bethesda, MD

- Introducing Innovation into Practice: Technical and Ethical Analyses of Preimplantation Genetic Diagnosis and Intracytoplasmic Sperm Injection Technologies, June 19-20, 1997, Bethesda, MD
- 26th Annual Meeting of Directors of NICHD Reproductive Sciences Research Centers, University of Washington, June 23, 1997, Seattle, WA
- 27th Annual Meeting of Directors of NICHD Reproductive Sciences Research Centers, University of North Carolina, Chapel Hill, May 11-12, 1998, Chapel Hill , NC
- Frontiers in Reproduction Research : A Legacy Symposium, Marine Biological Laboratories, June 23-27, 1998, Woods Hole, MA

LIAISON ACTIVITIES

- International and National: WHO Steering Committee, Task Force on Vaccines for Fertility Regulation, Geneva, Switzerland; Board of Directors, Burroughs Wellcome-NICHD Frontiers in Reproduction Training Course, Marine Biological Laboratories, Woods Hole, MA; PCOS Advisory Board Steering Committee, Park-Davis Women's Health Care, Divisions of Warner-Lambert Co., Morris Plains, NJ; PCOS Clinical Trials Consultation Panel, Bristol-Meyers Squibb Co., Princeton, NJ; Indo-U.S. Joint Working Group, Collaboration Program on Contraceptive and Reproductive Health, Governments of India and United States of America; Contraceptive Research and Development Program, U.S. Agency for International Development (Technical Advisory Committee and Immunocontraception Working Group)

- NIH: Electronic Administration Project Working Group; Working Group on Evaluating the Division of Research Grants; Human Embryo Research Panel Steering Committee; Interagency Working Group on Assisted Reproductive Technology; Human Radiation Studies Task Force; Evaluation Group:Guidelines for Addressing Scientific Irregularities in Grants and Contracts; Extramural Staff Training and Development Committee/Mentoring Program; Board of Directors, Grants Associate Program; Board of Directors, Extramural Associates Program; Program Officials/Project Officers Forum; Trans-NIH Research Animal Coordinating Committee; Neuroscience Steering Committee; Trans-NIH Endocrinology Research Working Group; Management Assessment Panel for Risks Assessment for NIH Cooperative Agreements; Kidney, Urologic and Hematological Diseases Interagency Coordinating Committee, Urology Subcommittee; Coordinating Committee for Research on Women's Health; Office for Research on Women's Health Working Group on Reproductive Health

- NICHD: Organizational Development Initiative-Task Groups on Program Planning and Program Organization; AIDS Research Planning Committee; Large Grant Policies Committee; Small Grants Policy Committee; EEO Advisory Committee; Discretionary Zone Funding Plan Implementation Committee; Referral Guidelines Committee; Minority and Disability Supplement Committee; Training Policy Committee

- Society for the Study of Reproduction: Membership Committee; Public Affairs Committee; Training Committee; Minority Affairs Committee

- American Andrology Society: Student Affairs Committee; Awards Committee; Nominating Committee; Andrology News from the NICHD Newsletter Column

- American Society for Reproductive Medicine; From the NICHD Newsletter Column

- American Society for Reproductive Immunology; From the NICHD Newsletter Column

PROGRAM ANNOUNCEMENTS (PAs) AND REQUESTS FOR APPLICATIONS (RFAs)

RFA: HD-94-015	Idiopathic Male Infertility
RFA: HD-94-016	Cooperative Multicenter Reproductive Medicine Network
RFA: HD-95-003	Specialized Research Center Programs or Center Core Grants to Support Research in Reproduction
PA: 95-006	Biology of the Menopause: Change of Ovarian Function
RFA: HD-95-008	Cooperative Specialized Infertility Research Center Program
RFA: HD-95-014	Nonhuman In Vitro Fertilization and Preimplantation Development
RFA: HD-95-017	Specialized Research Center Programs or Center Core Grants to Support Research in Reproduction
RFA: HD-95-018	Markers of Uterine Receptivity for Blastocyst Implantation
RFA: HD-96-008	Specialized Cooperative Centers Program in Reproduction Research
PA: 96-062	Research on HIV Infection in the Genitourinary Tract
RFA: HD-97-004	Male Germ Cell Growth and Differentiation
RFA: HD-97-008	Specialized Cooperative Centers Program in Reproduction Research
RFA: HD-98-004	Women's Reproductive Health Research Career Development Centers

COVER JOURNAL PUBLICATION ACKNOWLEDGMENTS- By Permission of the Publishers

Two recent instances (not pictured) appear in the May 1998 issue (v. 125, no. 10) of the journal "Development" in acknowledgment of contributory work by Christopher Bazinet and coworkers (R15 HD 31693) and the July 1998 issue (v. 12, no. 7) of the journal " Molecular Endocrinology" acknowledging the work of JoAnne Richards and coworkers (R01 HD 16272). Dr. Bazinet's cover is the first such honor for an Academic Research Enhancement Award (AREA) project in our program. Dr. Richard's cover represents a contribution by a recent NICHD Method to Extend Research In Time (MERIT) awardee.

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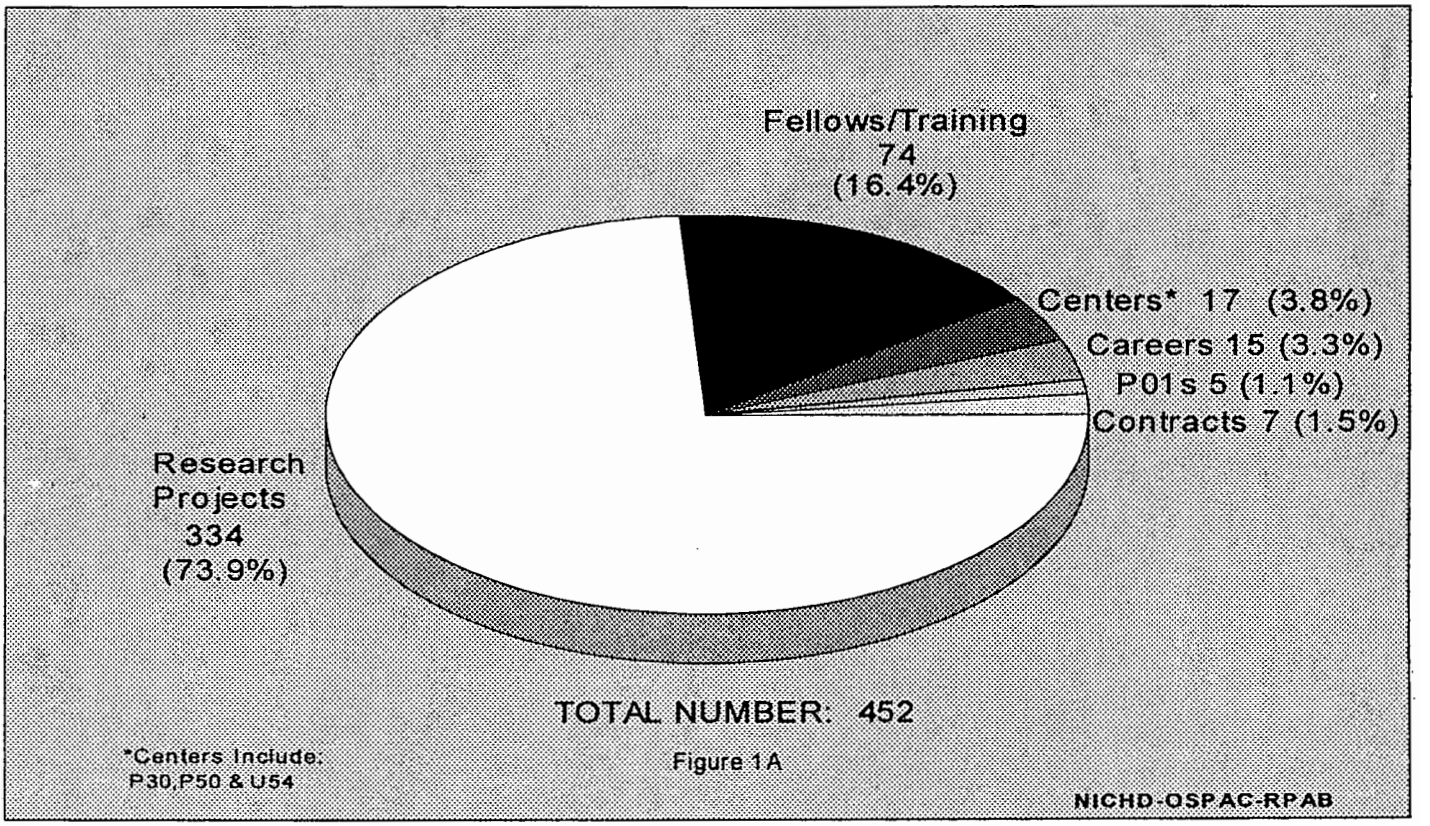
Baby Image (1994), Genetic Engineering News, Larchmont, N.Y. (Photographer, Stephen Marks, The Image Bank, N.Y., N.Y.)

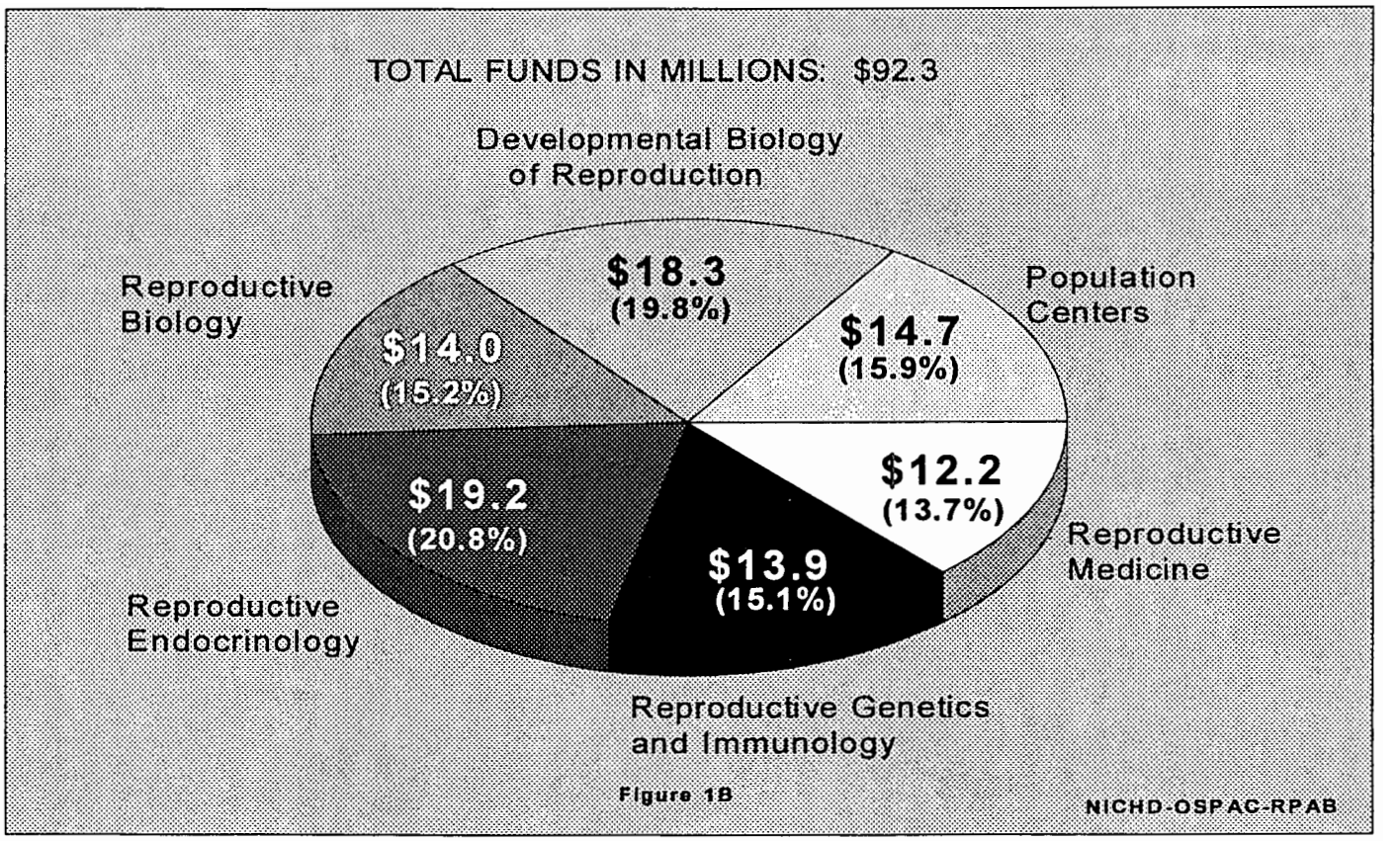
FISCAL DATA SUMMARIES FOR THE REPRODUCTIVE SCIENCES BRANCH

Data and figures prepared by RPAB, OSPAC, NICHD

REPRODUCTIVE SCIENCES BRANCH, FY 1997
NUMBER & PERCENT OF GRANTS & CONTRACTS

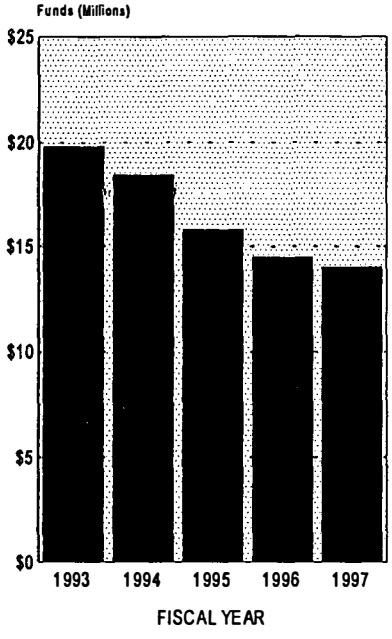


GRANTS & CONTRACTS BY RESEARCH CATEGORY

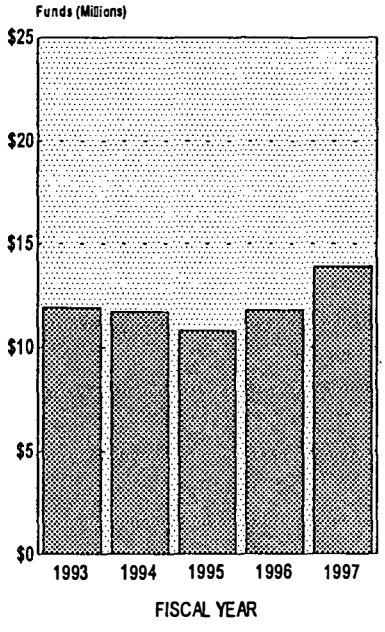


REPRODUCTIVE SCIENCES BRANCH
FUNDS FOR RESEARCH CATEGORIES
FY 1993 - FY 1997

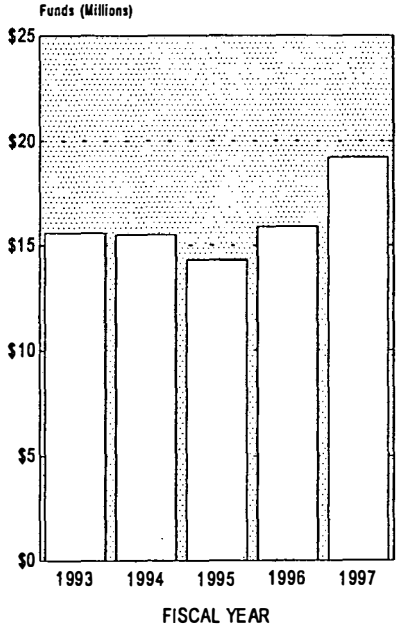
REPRODUCTIVE BIOLOGY



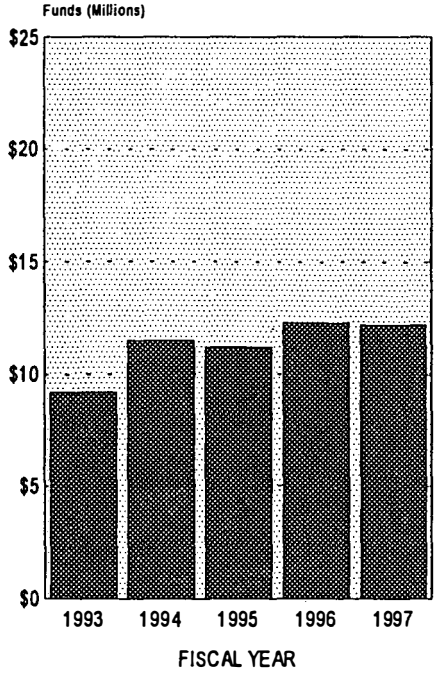
REPRODUCTIVE GENETICS AND IMMUNOLOGY



REPRODUCTIVE ENDOCRINOLOGY



REPRODUCTIVE MEDICINE



DEVELOPMENTAL BIOLOGY OF REPRODUCTION

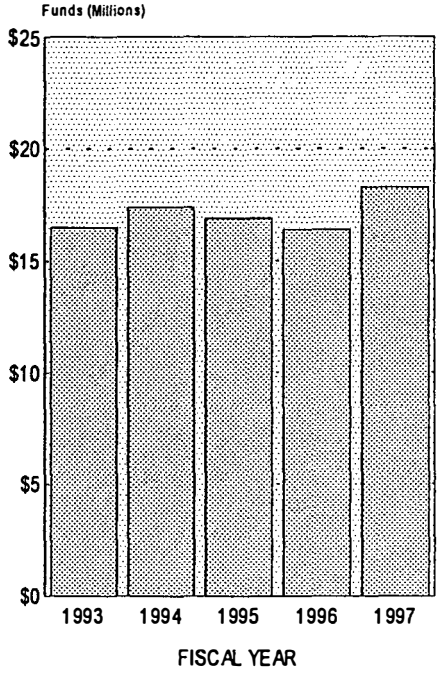


Figure 2
NICHD-OSPAC-RPAB

REPRODUCTIVE SCIENCES BRANCH, FY 88-FY 97

FUNDS IN CURRENT AND CONSTANT DOLLARS

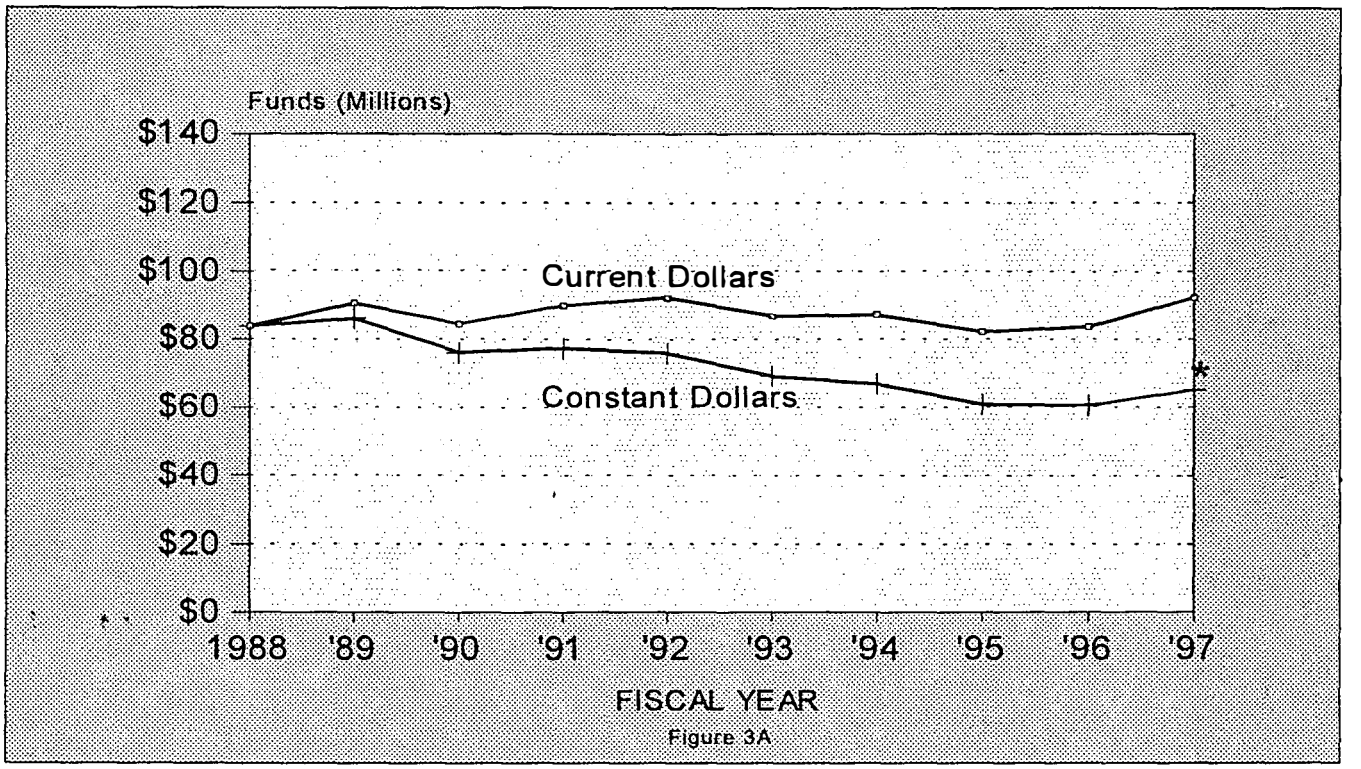


Figure 3A

* Constant Dollars (1988=100)

AVERAGE ANNUAL R01 GRANT COST

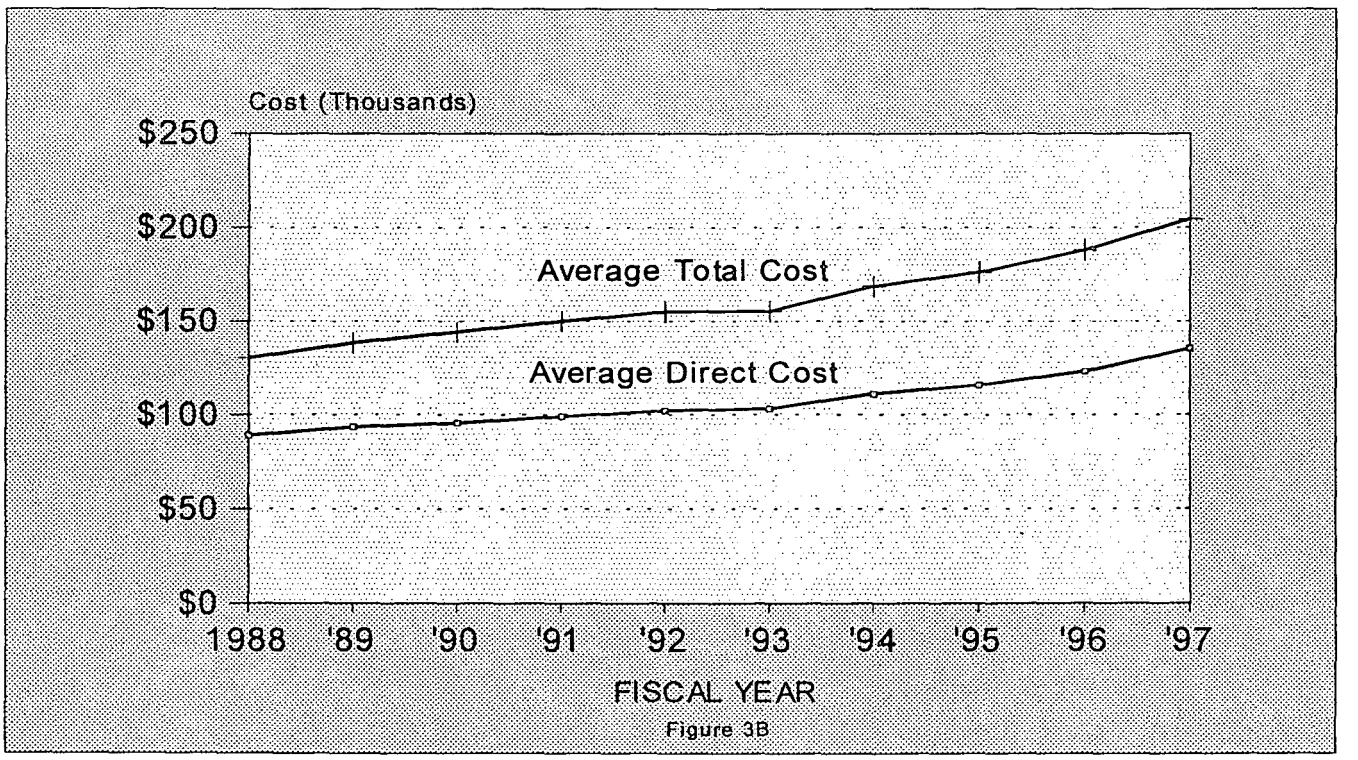


Figure 3B

REPRODUCTIVE SCIENCES BRANCH, FY 88-FY 97

NUMBER OF FUNDED GRANTS, BY MECHANISM

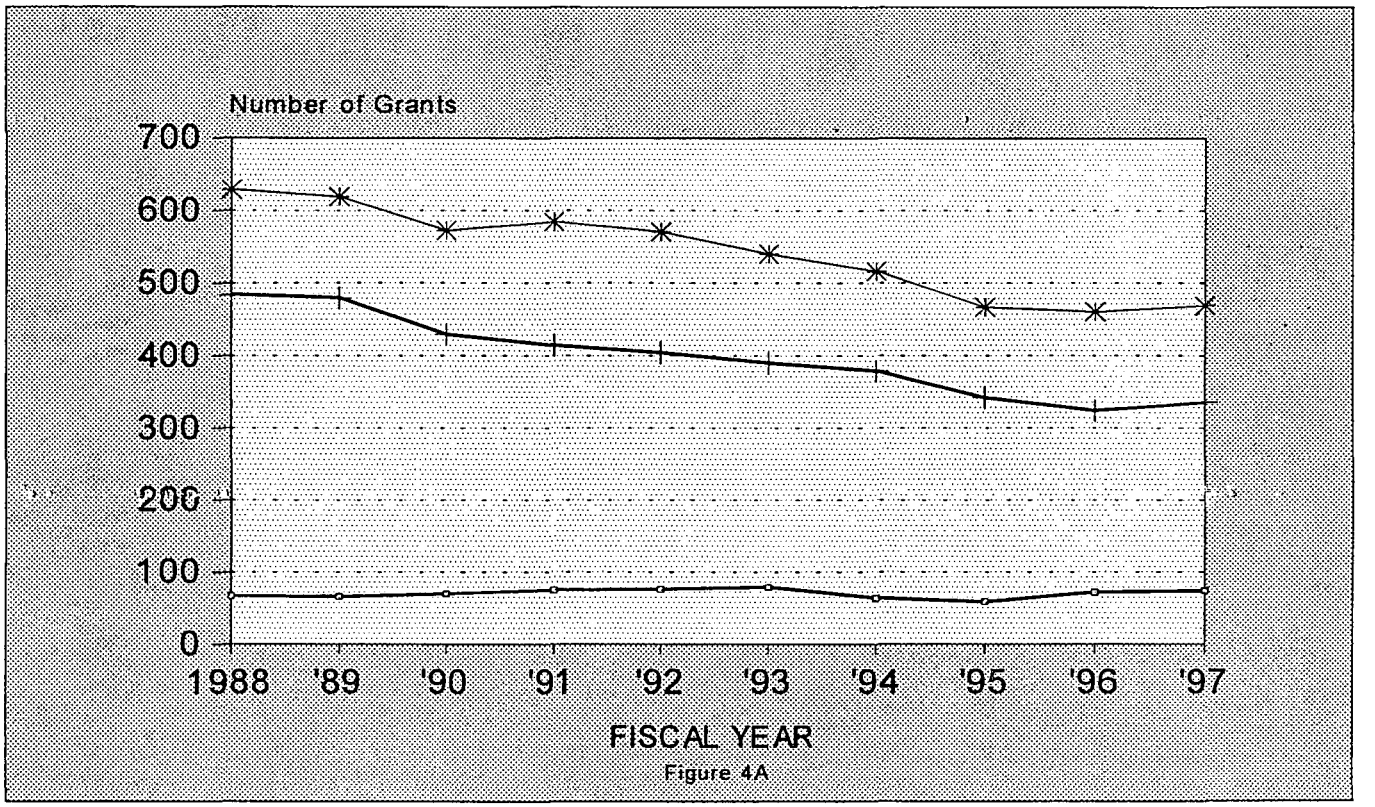


Figure 4A

F & T Research Total

FUNDS, BY MECHANISM

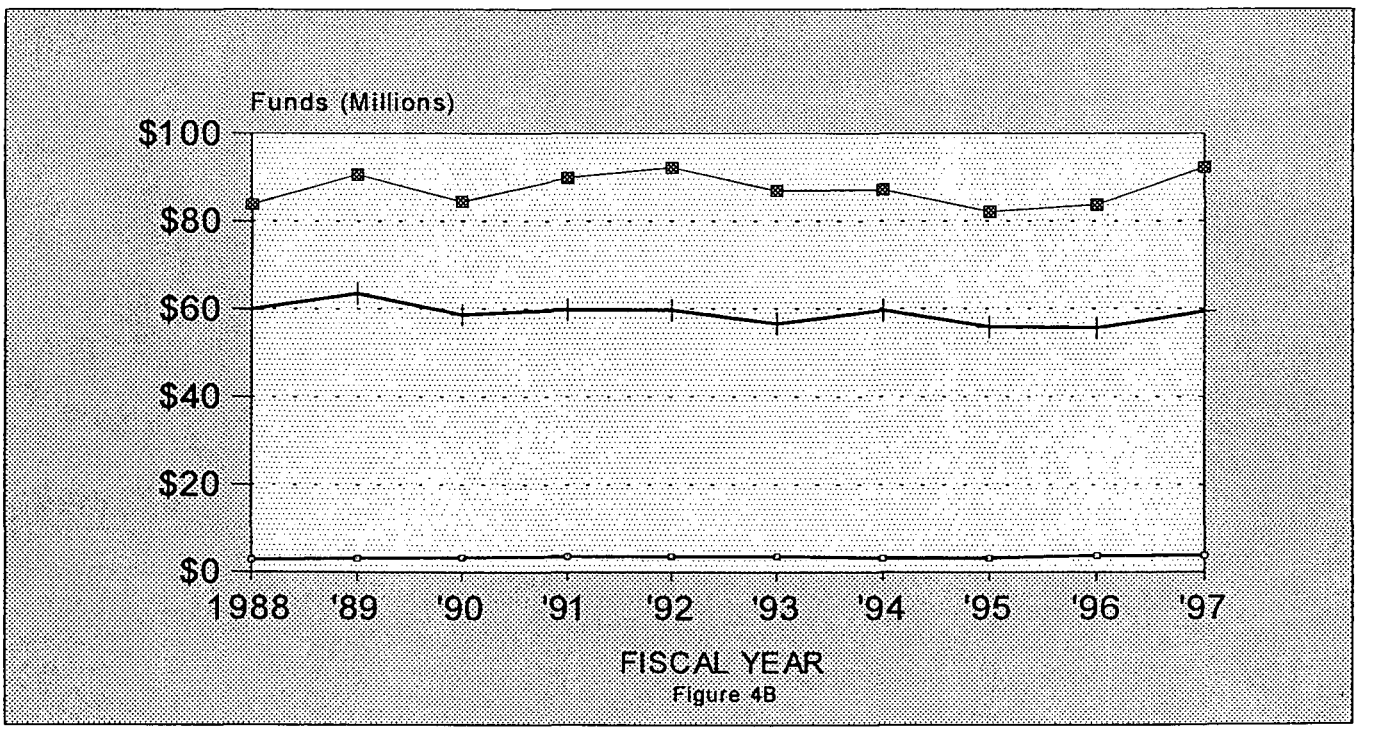


Figure 4B

F & T Research Total

NICHD-OSPAC-RPAB

REPRODUCTIVE SCIENCES BRANCH FY 88 - FY 97

NUMBER OF FUNDED GRANTS, BY MECHANISM

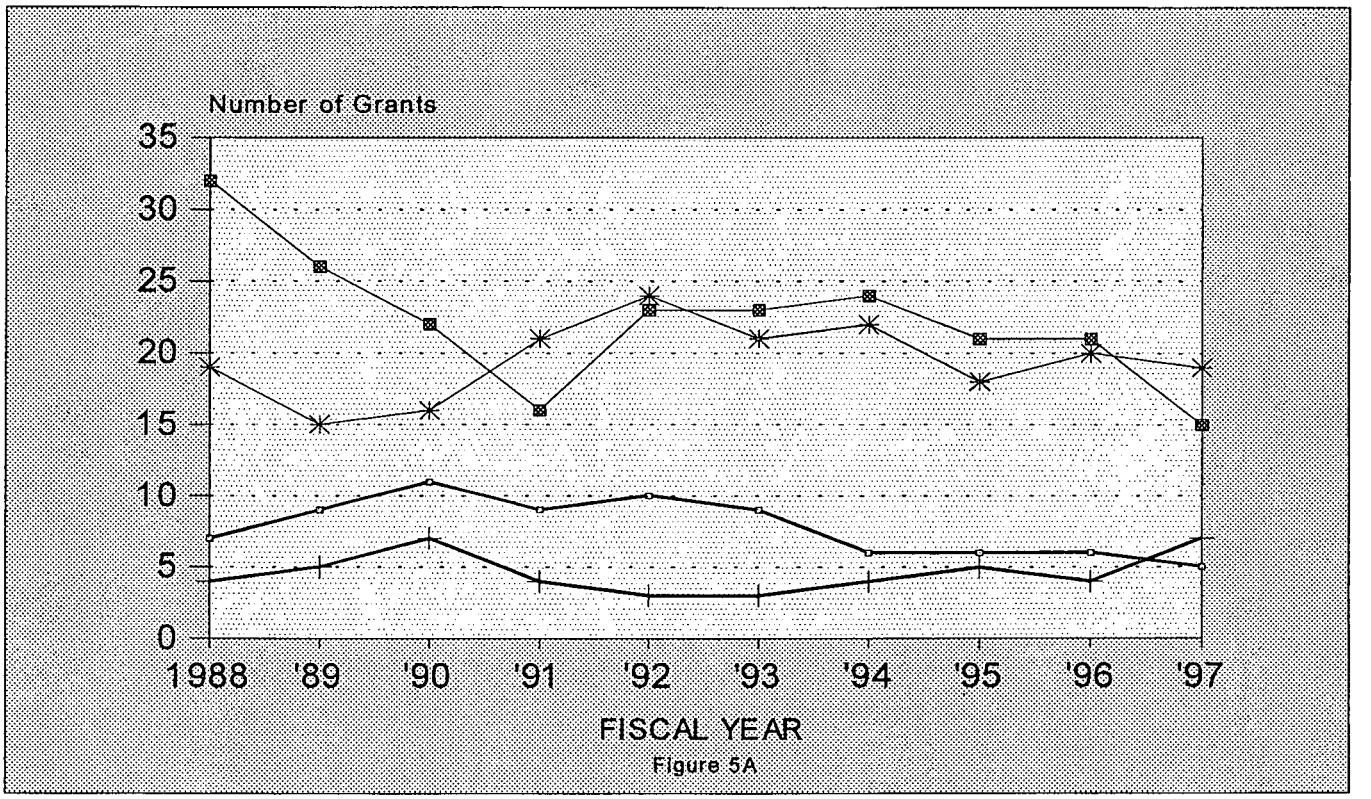


Figure 5A

FUNDS, BY MECHANISM

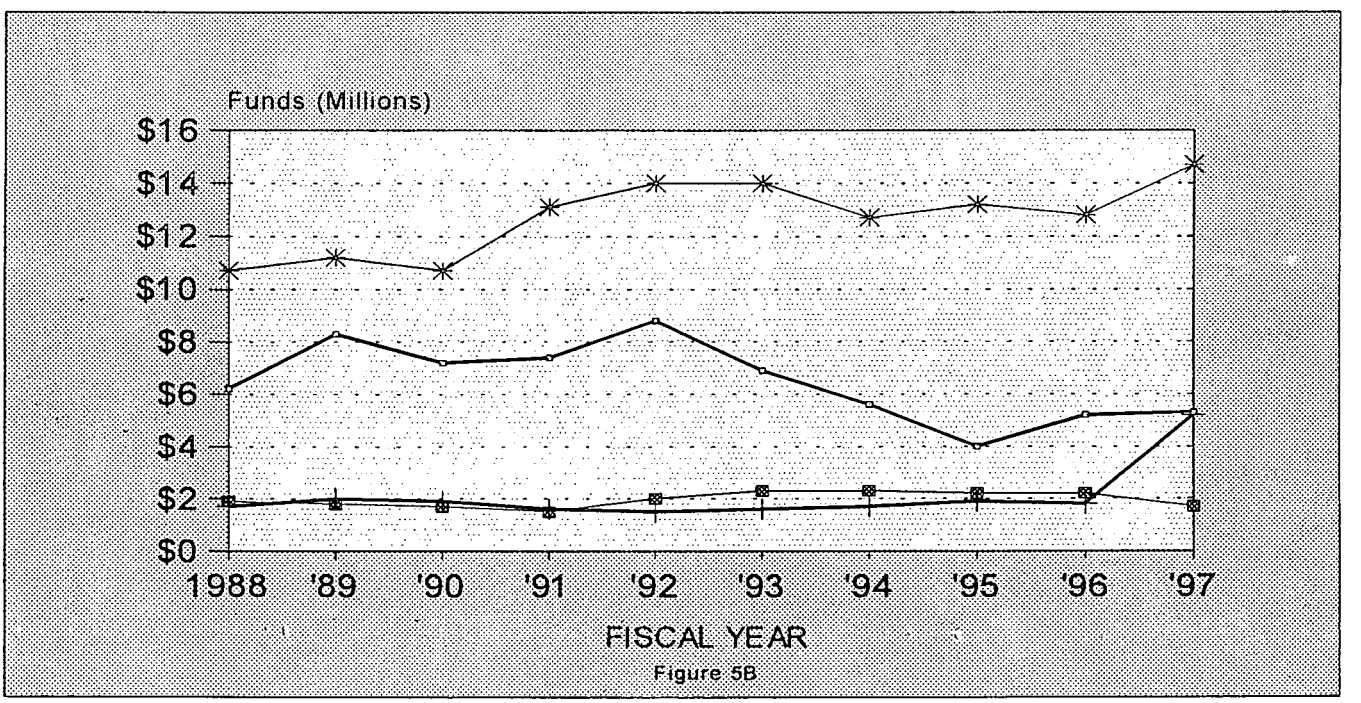


Figure 5B

NICHD-OSPAC-RPAB