

Reproductive Sciences Branch NICHD



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EXECUTIVE SUMMARY

The cycle of life hinges on reproduction, as each generation develops and matures to produce the succeeding generation. While it is obvious that reproductive health influences the quality of gametes, the research community is beginning to realize that the quality of gametes, in turn, influences the long-term health of the next generation. Reproductive health is not only the key to the continuity of life, but also to the quality of life.

Likewise, the challenge to completely understand the complex process of reproduction is also a cycle, as each answered question increases knowledge and simultaneously opens new areas to be explored. The mission of the Reproductive Sciences Branch (RSB) is to encourage and support scientific research aimed at alleviating human infertility and reproductive disorders, identifying new contraceptive leads, and expanding fundamental knowledge of the processes that underlie the success or failure of human reproduction. To achieve its mission, the RSB supports a comprehensive program of basic, clinical, and translational studies to increase understanding of normal reproduction and reproductive pathophysiology, and to develop more effective strategies for diagnosing, treating, and preventing conditions that compromise reproductive health. The Branch uses a variety of funding mechanisms, including investigator-initiated grants, cooperative centers, conference grants, as well as Requests for Applications (RFAs) and Program Announcements (PAs) to target neglected areas. The RSB also supports training and career development opportunities in the reproductive sciences for individuals at the undergraduate through post-graduate levels. Last year, the RSB proudly launched its expanded Web site, <http://www.nichd.nih.gov/cpr/rs/rs.htm>, to disseminate its mission, priorities, and activities to the scientific community and the public. This report, covering the Branch's activities from 1998-2002, summarizes RSB programs and presents highlights from some of the most significant and exciting research advances made by RSB-funded scientists.

The selected highlights of RSB-supported research that are presented here represent advances in knowledge of basic mechanisms of the reproductive process and provide steps toward promoting human reproductive health and eliminating reproductive disease. These highlights encompass technical, molecular, and clinical advances, as all three approaches are needed to advance the health and well being of the public. The advances are organized into four major topic areas: **Neuroendocrinology, Female Reproduction, Male Reproduction, and Developmental Biology of Reproduction.** The Branch also has programs for Reproductive Gynecology, Andrology, and Reproductive Genetics. The research advances of scientists in these fields are incorporated into the four sections listed above. The RSB is confident that these research highlights will contribute to the sense of awe and inspiration that reproductive science engenders.

Technological breakthroughs and the fast pace of scientific advances make this an exciting time for reproductive science. The Branch's plans for the future include expansion of the technological resources available for scientists in the field. DNA and protein microarray technology that assesses global transcriptional patterns and protein expression and the applications of these technologies to the study of reproduction and reproductive diseases are a high priority. Further development of reproductive genomic databases and tissue banks are also essential components of the Branch's future plans. Women's diseases, such as uterine leiomyoma, continue to be poorly understood, therefore the Branch will focus its energies in this

research area. Reproductive and gynecologic diseases of the post-pubertal adolescent have also come to the attention of the RSB as areas for research development. Further strengthening of the Andrology portfolio is an essential component of the Branch's strategic plan.

In addition, there is a pressing need to encourage young investigators to develop an interest in careers in the reproductive sciences. To that end, the RSB is considering ways to stimulate recruitment and retention of young scientists, especially those in underrepresented minorities, into the scientific community, while maintaining the recent gains in the education of physicians in women's health research. There is also a dearth of young scientists who are deciding on a career in men's reproductive health; thus, the RSB will make this area one of its top-priority training issues. Reproductive scientists with a PhD have stated a need for more exposure to clinical reproductive medicine in order to facilitate translational research. Thus the RSB plans to promote extensive cross-fertilization between basic science and clinical science grantees, a process that, to some extent, is demonstrated by several RSB programs described in this report.

SPECIAL PROGRAM INITIATIVES

SPECIALIZED COOPERATIVE CENTERS PROGRAM IN REPRODUCTION RESEARCH (SCCPRR)

Established in 1998, the SCCPRR is a research-based centers program that promotes multidisciplinary interactions between basic and clinical scientists. The ultimate goal of the program is to improve human reproductive health through accelerated, bi-directional transfer of knowledge between basic science laboratories and clinical care facilities (for more information go to <http://reprobio.stanford.edu/sccprnet/index.html>). The SCCPRR is funded through the U54 cooperative mechanism, which provides support for research projects and core services. As a cooperative agreement, center investigators work with staff from the National Institute of Child Health and Human Development (NICHD) to facilitate research collaborations within and between centers, as well as with private foundations and industry. The U54 mechanism provides more flexible administrative policies to meet the program's needs, greater program relevancy to the NICHD mission, and a clear means for performance-based budgeting decisions by the NICHD and the SCCPRR Steering Committee. Currently, there are 14 SCCPRR centers: Baylor College of Medicine; University of Kansas; Johns Hopkins University; University of Maryland; Massachusetts General Hospital; University of North Carolina; Oregon Health Sciences University; University of Pittsburgh; Stanford University; University of Virginia; University of California, San Diego; University of Washington; University of Illinois at Chicago; and Vanderbilt University.

To promote and facilitate interactions among centers, the SCCPRR established research focus groups, which include investigators from different centers, in the areas of male reproduction, endometrial biology, ovarian physiology, and neuroendocrinology. These groups meet twice each year to exchange information and form collaborations. Collaborating investigators may submit Collaborative Research Initiatives (CRIs) on topics related to their research that is supported by an SCCPRR award. These initiatives require the expertise of several centers, and

usually have a clinical/applied focus. Three such initiatives have been or are currently being supported through administrative supplements; two of these initiatives are co-funded by the NICHD and the Office of Research on Women's Health (ORWH).

A focus group was established to monitor the SCCPRR's needs for computer technology and special resources. In response to the group's recommendations, human tissue banks were established at Stanford University, the University of North Carolina, Johns Hopkins University, and the University of California, San Diego; and, a non-human primate tissue bank was established at the Oregon National Primate Research Center of the Oregon Health Sciences University. An online system, the Reproductive Tissue Sample Repository, has also been developed; this system provides SCCPRR investigators with real-time access to tissue inventories and will allow online requests. For expression profiling, SCCPRR investigators have free-of-charge access to the microarray facility at the University of Washington, while the University of Maryland's Immunocytochemistry and *In Situ* Hybridization Core offers laser capture microdissection.

The SCCPRR also provides resources for the general scientific community, such as a collection of cDNA libraries from reproductive tissues of rats, mice, monkeys, and humans. This activity is supported through an intra-agency agreement with the National Cancer Institute (NCI) Cancer Genome Anatomy Project. The SCCPRR supports the development and maintenance of online gene/protein databases for the ovary (<http://ovary.stanford.edu/>), male reproductive tissues (<http://mouse.genetics.washington.edu/>), and the endometrium (in development).

NATIONAL COOPERATIVE PROGRAM FOR INFERTILITY RESEARCH (NCPIR)

The NCPIR was formed in response to a congressional recommendation for infertility research centers to support research on the development of new diagnostics, therapeutics, and cures for the detection, management, and alleviation of human infertility. Current awardees include: the University of Pennsylvania, which is focusing on polycystic ovarian syndrome (PCOS); and the Massachusetts General Hospital, which is focusing on the hypothalamic-pituitary-gonadal axis, including the role of inhibins as markers of ovarian function, and on the molecular biology of gonadal development. In addition, the two centers presently collaborate on genotype-phenotype studies in PCOS. All NCPIR investigators have access to SCCPRR-supported resources and can collaborate with SCCPRR investigators on CRIs.

SPECIALIZED COOPERATIVE REPRODUCTIVE SCIENCE RESEARCH CENTERS AT MINORITY INSTITUTIONS

The ultimate goals of reproductive science research supported by the NICHD are to promote basic science and to develop new knowledge that leads to clinical applications that improve reproductive health. One way the Branch is supporting this effort is by increasing the number of new and experienced minority investigators in reproductive science. In 2000, the NICHD formed a cooperative program to stimulate the development of a competitive research environment in reproductive science at minority institutions, where such research remains

relatively unexplored. Reproductive scientists at minority institutions were invited to establish a collaborative partnership with one of the NICHD-designated reproductive science research institutional programs, thereby benefiting from a broader range of resources, experienced leadership, and exposure to ongoing research projects. Such centers bring together strong teams of experienced and new investigators focused on a reproductive science theme to share essential facilities, services, knowledge, and other resources. The Morehouse School of Medicine, in partnership with the University of Pittsburgh, received the first award in September 2001.

NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK (RMN)

The RMN was established in 1990, to conduct large, multi-center clinical trials in female and male infertility and reproductive diseases and disorders. Major changes occurred in the RMN over the past five years, including its second recompetition in 1999. As a result, three continuing centers (denoted by *), five new centers, and a Data Coordinating Center (DCC) were funded: *Baylor College of Medicine; *University of Pennsylvania; *University of Alabama; Pennsylvania State University Medical School; University of Colorado; UMD-New Jersey Medical School; University of Texas, SW; and Wayne State Medical School. The DCC is at Duke University.

A new infrastructure was established for the RMN. The Steering Committee, which consists of the principal investigators of each RMN site, the NICHD research coordinator, and an independent NICHD-appointed chairperson, makes consensus decisions regarding the design and implementation of study protocols. In addition, the RMN now includes a Clinical Trials Advisory Board that reviews proposed protocols, suggests revisions to those projects, and advises the Steering Committee on new research endeavors. The board is composed of individuals with expertise in biostatistics, epidemiology, reproductive endocrinology, and general obstetrics and gynecology who are appointed by the NICHD; the NICHD research coordinator and the Steering Committee chair are ex-officio board members. The RMN also includes a Data Safety and Monitoring Committee that is convened by the NICHD to review clinical protocols and make recommendations at the inception of each protocol, as well as during the trials. The DCC initiated a Web site for the RMN that provides secure access for RMN investigators, as well as public access (<http://rmn.dcri.duke.edu>). Lastly, the RMN transitioned to a new budget process that includes a base for each center's fixed costs and a capitated budget for each protocol.

NATIONAL COOPERATIVE PROGRAM ON NONHUMAN *IN VITRO* FERTILIZATION AND PREIMPLANTATION DEVELOPMENT (NCPIV): 1986-2000

In 1986, the ability to culture oocytes and preimplantation embryos was severely limited. The NICHD established the NCPIV, a research collaboration whose primary goal was to improve the culture conditions for mammalian oocyte and preimplantation embryo development, to address this problem. Technical and conceptual results from the program have appeared in more than 250 publications. The results have been widely used to improve culture conditions in both basic

and applied research on laboratory rodents, nonhuman primates, and farm animals, and have even translated to improved conditions in human infertility clinics.

NATIONAL COOPERATIVE PROGRAM ON MARKERS OF UTERINE RECEPTIVITY FOR BLASTOCYST IMPLANTATION (NCPMURBI): 1993-2001

This cooperative program was established to characterize the uterine tissues that are receptive to the blastocyst at the time of implantation. After two consecutive four-year project periods, the program concluded in the spring of 2001. During the second four-year period, NCPMURBI participants published more than 100 articles arising from their research. Toward the end of the final grant period, the NCPMURBI held a meeting to review progress and to establish future directions. The meeting summary was published in *Trends in Endocrinology and Metabolism* (11:116-118, 2000). The members of this group also wrote a review on blastocyst implantation for *Developmental Biology* (223:217-237, 2000).

COOPERATIVE PROGRAM ON TROPHOBLAST-MATERNAL TISSUE INTERACTIONS (CPTMTI)

This program, initiated in April 2002, addresses clinically important topics, such as pregnancy failure, ectopic pregnancy, and abnormal placentation associated with pre-eclampsia. The CPTMTI has five sites: University of California, San Francisco; Stanford University; Baylor College of Medicine; University of Delaware; and State University of New York at Stony Brook. The participating scientists are investigating the roles of selected molecules at the interface between trophoblast and maternal tissues during early pregnancy. This program will continue for the next five years.

RSANET (REPRODUCTIVE SCIENCES OF THE AMERICAS NETWORK) PROJECT

The RSANET project is an international training and research network of reproductive scientists in Latin American member countries. Monthly electronic reports enhance communication among the investigators and allow them to announce conferences and research opportunities. The Americas Fellowship program of RSANET supports training of postdoctoral investigators from Latin American member countries at leading North American institutions for up to 24 months. The Americas Fellowship program is co-funded by the NICHD and the Fogarty International Center at the National Institutes of Health (NIH), through a supplement to the D43 mechanism for International Training and Research in Population and Health. Currently, the young scientists selected have begun training at the institution of their choice.

CONTRACEPTION AND INFERTILITY RESEARCH LOAN REPAYMENT PROGRAM (CIR-LRP)

In 1997, the NICHD announced its CIR-LRP for forgiveness of educational loan debt for qualified health professionals (including graduate students) who commit to conducting research on contraception and/or infertility for at least two years. Eligible sites for participation include

Cooperative Specialized Contraception Research centers, Cooperative Specialized Infertility Research centers, SCCPRR sites, RMN sites, Contraceptive Clinical Trials Network sites, Women's Reproductive Health Research Career Development centers, and the NIH Intramural Program. Fellows of the Reproductive Scientist Development Program (RSDP) are also eligible to participate in the program. Through fiscal year 2001, the CIR-LRP executed 36 initial contracts and four, one-year extension contracts for a total obligation (debt repayment plus tax liability) of \$2,348,385.

THE EXTRAMURAL ASSOCIATES (EA) PROGRAM

The EA program was established in 1978, under the auspices of the NIH's Office of Extramural Research, to facilitate the participation of underrepresented minority and women's institutions in biomedical and behavioral research and research training. The program provides NIH residency training to representatives from EA-eligible institutions. In June 2001, the EA program moved to the NICHD and has served as the centerpiece for the NICHD's Health Disparities Initiative since that time. The program is run by Dr. Matthew Kinnard and is administered through the RSB. In 1994, the EA program was augmented with the Extramural Associates Research Development Award (EARDA), a three-year grant to support infrastructure development or expansion for a sponsored program at the EA fellow's institution. Since the inception of the EARDA grant, more than 50 individuals from EA-eligible institutions have been trained and received the EARDA grant.

PROGRAMS TO SUPPORT RESEARCH BY MINORITIES AND PERSONS WITH DISABILITIES

The RSB uses several additional tools to increase the participation of underrepresented groups in research and research training. For training grants, there is a firm policy that institutional training programs propose and carry out a plan for recruitment of minorities; these plans are rigorously monitored for all new, competing, and non-competing applications. F31 pre-doctoral fellowships support eligible candidates from minority groups or those with disabilities. Supplemental funds are available to support minority high school, undergraduate, and graduate students, as well as postdoctoral fellows and new investigators. Through supplements to existing research grants, the RSB supported nine minority students in fiscal year 1999, five in fiscal year 2000, seven in fiscal year 2001, and 10 in fiscal year 2002. The Branch administers the EARDA program for the development of research infrastructure at minority institutions (see above). Finally, the RSB issued an RFA titled *Cooperative Reproductive Science Research Centers at Minority Institutions* (HD-00-019; see page 3).

The remainder of this report highlights research advances from the RSB and Branch-supported scientists.

NEUROENDOCRINOLOGY

The pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), regulate the production of steroid hormones and mature gametes by the male and female gonads. Both LH and FSH are released episodically in response to the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Deviations in the timing of GnRH pulses, and consequent timing of LH and FSH pulses, can result in infertility. Because LH and FSH are critical to normal reproduction, multiple levels of control govern their release. The amount of LH and FSH released during each secretory episode depends on the amount of GnRH released, and on the ability of the pituitary gland to respond to GnRH. Sex steroids from the gonads can alter both the amount of GnRH released, and pituitary responsiveness to GnRH. Therefore, it is essential to understand the biochemical and molecular processes that regulate pulsatile secretion of GnRH and responsiveness of the pituitary gonadotropes to LH and FSH.

MIGRATION OF GnRH NEURONS DURING DEVELOPMENT

During development, GnRH neurons migrate from the olfactory placode to their final residence in the forebrain. Kallman's syndrome, a disorder associated with irreversible puberty delay, infertility, and low serum gonadotropin levels, results from failure of the GnRH neurons to migrate properly. A study that compared a GnRH cell line derived from migratory GnRH neurons in the olfactory area with another derived from post migratory GnRH neurons determined that the gene encoding adhesion related kinase (Ark) is exclusively expressed in migratory GnRH neurons. The ligand for Ark, growth arrest-specific gene 6 (Gas6), is co-expressed with Ark in this subset of GnRH neurons. Interestingly, GnRH gene expression is repressed during neuronal migration, and this repression appears to be mediated by Ark. Additional work identified several novel factors involved in the Gas6/Ark signaling cascade that prevent GnRH synthesis during the period of neuronal migration. Misexpression of one or more of these factors may contribute to the failure of GnRH neurons to migrate, resulting in hypogonadotropic hypogonadism and infertility.

INTRINSIC CONTROL OF GnRH PULSATILITY

One of the remaining and perhaps most difficult challenges facing researchers in the field of reproduction is the elucidation of the intrinsic cellular and molecular mechanisms that govern the pulsatile release of GnRH from individual neurons. Optimum fertility depends on properly timed pulses of GnRH secretion. Thus, to combat neuroendocrine-based infertility, it is essential to understand the mechanisms of pulsatility. A study that tested the hypothesis that pulsatile GnRH release is linked to the regulation of intracellular levels of the signaling molecule, cyclic adenosine monophosphate (cAMP), made significant progress toward defining the molecular and cellular bases of pulsatile GnRH secretion. The data demonstrated that inhibiting enzymes that degrade cAMP, which would have the effect of increasing cAMP levels, resulted in marked stimulation of GnRH release from GnRH clonal cells.

In other exciting studies conducted on individual clonal GnRH cells, three important discoveries were made. First, bursts of GnRH secretion were linked to changes in intracellular calcium levels, but not all calcium oscillations resulted in GnRH secretory episodes. Second, neighboring cells synchronize their release of GnRH by communication through both calcium channels and gap junctions. Finally, GnRH exocytotic pulses trigger GnRH gene expression, ensuring that GnRH neurons are always ready to respond to changes in the reproductive axis.

While the availability of clonal cell lines greatly facilitates progress in GnRH neurobiology, it is important to note that these cells lack the normal synaptic inputs and transcription factors that regulate GnRH expression. Transgenic mice whose GnRH neurons are marked with a fluorescent protein provide a much-needed model to study live GnRH neurons. In contrast to the results from cell lines, data from these mice suggest that gap junctions play only a minor role in synchronizing GnRH neurons.

GENE EXPRESSION OF GnRH IN THE HYPOTHALAMUS AND OVARY

GnRH is specifically expressed only in certain hypothalamic neurons, and in some peripheral reproductive tissues. Correct expression of GnRH is paramount to normal reproductive function, but had been difficult to study *in vivo*. To learn more, one study used lines of transgenic mice that carried different sized fragments of the mouse GnRH (mGnRH) gene promoter fused to a reporter gene to demonstrate which specific regions of the promoter stimulate GnRH expression. The results showed that a region in the proximal promoter directs mGnRH gene expression to both the ovary and hypothalamus. A putative enhancer farther upstream may bind specific hypothalamic proteins to increase mGnRH expression. An upstream region appears to mediate repression of mGnRH expression in the ovary. This study was the first to define a region of the GnRH promoter that directs GnRH expression in both neural and non-neural tissues *in vivo*. Further studies are needed to elucidate the specific mechanism by which hypothalamic and ovarian GnRH expression are differentially regulated.

NOVEL GnRH AND GnRH RECEPTORS

For many years, researchers thought only one type of GnRH was present in mammalian brains, despite the fact that 14 additional forms of GnRH were identified in the brains of lower vertebrates. Recently a second form of GnRH (GnRH-II) was discovered in high levels in the brains of human and non-human primates. Studies have now shown that GnRH-I and GnRH-II are expressed in distinct locations, which suggests that the two peptides have different functions and regulatory mechanisms.

In addition, researchers cloned the type II GnRH receptor gene from rhesus monkeys. The ubiquitous expression of GnRH-II and its receptor suggests that many effects originally attributed to GnRH-I may, in fact, be due to GnRH-II. Work to distinguish the relative roles of the two GnRH peptides in mammalian reproduction awaits the design of type-specific GnRH antagonists. Results of such work could lay the foundation for novel approaches to contraceptive development, as well as for the development of novel treatments for human infertility.

GnRH RECEPTOR MUTATIONS

Idiopathic hypogonadotropic hypogonadism (IHH) is one of the most common causes of hereditary hypogonadism. Cases of IHH can be sporadic or familial, with either X-linked, or autosomal inheritance, indicating that several genes influence this phenotype. GnRH receptor mutations are the most commonly identified causes of autosomally inherited IHH. In males, incremental decreases in GnRH binding and/or post-receptor signaling deficits result in a broad spectrum of IHH phenotypes, including variations in gonadotropin levels and testes size. Researchers hypothesized that individuals with milder forms of IHH, such as those with fertile eunuch syndrome, may harbor partially inactivating defects in the GnRH receptor. In support of this hypothesis, scientists identified a novel mutation in the GnRH receptor gene from an individual with the fertile eunuch syndrome whose symptoms were reversed by treatment with human chorionic gonadotropin (hCG).

DISCOVERY OF INHIBIN-BINDING PROTEINS

Activin and inhibin are dimeric proteins that stimulate and inhibit, respectively, FSH secretion from the anterior pituitary gland. A number of type I (signaling) and type II (binding) receptors for activin, identified in the 1990s, are responsible for mediating the effects of activin on cellular function. In general, inhibin antagonizes the effects of activin. This antagonism was thought to occur through competition between activin and inhibin for access to dimerization partners and for binding to the activin receptor. However, other evidence suggested the existence of a separate inhibin receptor.

Recently, two independent laboratories identified membrane components that can bind inhibin. One study reported that betaglycan, a receptor for transforming growth factor- β (TGF- β), also binds inhibin with high affinity and enhances the ability of inhibin A to bind to the activin type II receptor. In the other study, a novel inhibin binding protein, called InhBP, was cloned. InhBP mRNA levels vary during the rat estrous cycle; these levels are inversely correlated with serum FSH levels, and positively correlated with serum inhibin B, but not inhibin A levels. Additional studies demonstrated that inhibin B, but not inhibin A, acts through InhBP to antagonize activin action. Thus, the two forms of inhibin, once thought to be interchangeable, were shown to have discrete molecular functions.

EXERCISE AND REPRODUCTIVE FUNCTION

While the benefits of a moderate exercise regimen cannot be overstated, chronic, strenuous exercise can perturb normal reproductive function in women. The prevalence of menstrual acyclicity in female athletes (from 1 percent to 44 percent) greatly exceeds the prevalence in sedentary women (estimated at 2 percent to 5 percent). Milder abnormalities, such as luteal phase disturbances, occur in as many as 42 percent of women who exercise moderately. Unfortunately, because almost all studies in humans have been cross-sectional, what changes may have heralded the onset of frank reproductive dysfunction are unknown. In longitudinal studies of strenuous exercise in non-human primates, scientists found reduced serum LH levels

and luteal phase progesterone secretion, an extended follicular phase, and an abrupt transition to amenorrhea (lack of menstruation). Feeding the amenorrheic monkeys supplemental calories reversed these changes. Interestingly, the transition to acyclicity was not correlated with initial body weight, training intensity, or food intake, but was correlated with levels of thyroid hormone (T_3), a marker of cellular energy availability. T_3 was low when amenorrhea was induced, but levels increased when amenorrhea was reversed.

STEROID REGULATION OF SEXUAL BEHAVIOR

Administration of estrogen increases sexual receptivity in female rats; similarly, testosterone regulates reproductive behaviors in male rats. The effects of estrogen are likely mediated through intracellular estrogen receptors (ER). Testosterone may act directly, by binding to androgen receptors, or indirectly, after it is converted into estrogen in brain and other peripheral tissues, by binding to ER. To determine which behavioral responses were dependent on the presence of ER, scientists tested the behavior of female and male mice lacking the ER α isoform. As expected, steroids did not induce sexual receptivity in ovariectomized female mice that lacked ER α . Furthermore, in comparison to normal females, the ER α -deficient female mice were more aggressive and displayed less parental behavior toward newborn pups. On the other hand, castrated male mice that lacked ER α responded to testosterone treatment with normal sexual behaviors, although they failed to ejaculate; the mice were less aggressive, but also less parental than normal male mice. These studies demonstrated gender differences in sexual behavior that are mediated by estrogen.

LEPTIN AND REPRODUCTION

Investigators at the SCCPRR center at the University of Washington conducted the first studies in non-human primates with the novel appetite-suppressing peptide, leptin. When fasted male monkeys were given leptin, the serum levels of the gonadotropins LH and FSH increased. These results suggest that, in primates, leptin is a metabolic signal to the reproductive axis.

FEMALE REPRODUCTION

The female reproductive system is dynamic, with cyclic fluctuations in the secretion of hormones from the brain, anterior pituitary gland, and the ovary. These hormones promote: the development of ovarian 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. To understand the physiology and pathophysiology of human reproduction, it is critical to delineate the mechanisms that govern this finely tuned sequence of hormone changes and their effects using various molecular, cell, and animal models.

The brain and pituitary comprise the neuroendocrine level of control (discussed previously), while the ovaries, uterus, oviducts, and vagina are collectively referred to as the reproductive tract. Much of the RSB-supported research on female reproduction addresses the actions and interactions of the neuroendocrine system and the reproductive tract. The pathophysiology and treatment of benign (non-cancerous) gynecologic disorders also are priority areas for the RSB. The Branch has developed several new initiatives to encourage the study of pelvic floor disorders and incontinence, vulvodynia, and endometriosis. The RSB portfolio in female reproduction includes studies in basic research, translational research, and clinical studies.

OVARIAN FUNCTION

Mechanism of gonadotropin action

In the testis and ovary, the gonadotropins LH and FSH bind to and activate their receptors. Activation of these receptors leads to synthesis of steroid hormones, and to the production and release of germ cells. Through means that remain enigmatic, each gonadotropin exerts its own specific action. Elucidation of structural differences between the gonadotropins may help explain their differential binding and effects. LH, FSH, and hCG have identical α subunits, but each has a unique β subunit. Research found that a portion of the β subunit, termed the “seatbelt,” wraps around the α subunit to stabilize the heterodimer. The unique “seatbelt” of each gonadotropin confers receptor specificity. In contrast to a previously proposed model, these results suggest that the seatbelt does not directly contact the receptor. Instead, it is now proposed that the groove between the α and β subunits binds to the receptor and transmits the hormone signal. Studies such as this one may drive the design of gonadotropin analogs and inhibitors that can be useful for basic science, and, potentially, in contraception or in the treatment of infertility.

The preovulatory surge of LH down-regulates the expression of LH receptor (LHR) in the ovary and also decreases LHR mRNA levels. Just prior to ovulation, LH mRNA is transcribed normally, but is subsequently lost. In rat and human ovarian cytosol, a protein named LHR binding protein (LRBP) specifically binds to the coding region of LHR mRNA and seemingly promotes its degradation. A recent study showed that the down-regulation of LHR is accompanied by increased binding between LRBP and LHR mRNA. During follicular maturation, ovulation, and luteinization, LHR mRNA expression inversely correlates with LRBP binding activity. Furthermore, LRBP accelerates the decay of LHR mRNA *in vitro*. For the first time, these observations supported the notion that cell-surface LHR availability is regulated through LHR mRNA degradation by LRBP.

Steroidogenesis and steroid hormone action

Through a series of enzymatically catalyzed steps, ovarian steroidogenesis converts cholesterol into estrogen (estradiol) and progesterone. These steroids are crucial for promoting and maintaining the female phenotype, normal ovarian function and ovulation, and support of the early stages of pregnancy. Researchers have known the basic regulation of ovarian steroidogenesis by LH and FSH for quite some time. However, new layers of control emerge as science delves further into this important process.

Steroid hormones induce their effects by binding to nuclear receptors. Steroid receptor co-activators interact with the bound nuclear receptors to mediate the transcriptional response of target genes. Steroid receptor coactivator-3 (SRC-3) is important in normal female puberty and reproduction. In mice, SRC-3 is highly expressed in the oocytes, mammary glands, hippocampus, olfactory bulb, smooth muscle, hepatocytes, and vaginal epithelium. SRC-3 knockout mice show growth retardation, delayed puberty, sub-fertility, and blunted mammary gland development. The SRC-3 knockout mice also had low levels of estrogen, which may explain the pubertal and reproductive phenotype, although the link between SRC-3 and estrogen production remains unclear. A close homolog, SRC-1, is expressed in different tissues, and SRC-1 knockout mice do not have a reproductive phenotype. These results clearly demonstrate that SRC-3 and SRC-1 have different physiological roles.

Follicle endowment

During embryonic life, the germ cells of mammals migrate into the gonad and begin to proliferate rapidly. In females, the extensive proliferation is followed by a dramatic decrease in the number of germ cells, probably through apoptosis (programmed cell death). Female mammals, including women, are thus born with a finite number of oocytes in primordial follicles (those present at the earliest phase of follicle development) that will only decrease over the lifetime. When the pool of primordial follicles has been exhausted through apoptosis, atresia, and the ovulation of the select few that mature to dominance, menopause ensues. Thus, the establishment and regulation of the primordial follicle pool are important regulators of reproductive potential.

The apoptosis pathway that depletes ovarian follicles is multifaceted. Scientists examined the role of ceramide metabolism on primordial follicle endowment. Ceramide is a cell death signal that is produced by sphingomyelinase; however, ceramide can also be metabolized to sphingosine-1-phosphate (S1P), which *inhibits* apoptosis. The balance between ceramide and S1P may determine cell fate. Ovaries of mice that lack the acid sphingomyelinase gene had significantly more primordial, primary, and preantral follicles, when compared to controls. The protective effects of sphingomyelinase disruption lasted at least until early adulthood, and numbers of both primordial and more mature follicles were increased. In contrast, mice engineered to over-express the cell survival factor *bcl-2* did not maintain their “extra” primordial follicles. Together these data suggest that removal of cell death signals, as opposed to expression of protective factors, is more important for the survival and maturation of ovarian follicles.

Chemotherapy or radiation treatments for cancer often induce oocyte apoptosis and may lead to the premature onset of menopause. Fertility-sparing treatments would have tremendous value for patients undergoing such treatments. Studies of ceramide metabolism also included a test of S1P as a protectant against radiation. S1P was injected into the ovarian bursae of mice before radiation exposure. In contrast to irradiated mice who did not receive S1P, the S1P-injected mice retained normal numbers of all follicle types and were able to produce healthy offspring. Thus, this class of small lipids may represent a new approach to preserving fertility in women threatened with premature ovarian failure (POF).

Folliculogenesis and selection of a dominant follicle

Primordial ovarian follicles mature through the primary and preantral stages, acquire an antral cavity, and develop into pre-ovulatory Graafian follicles. The classic endocrine paradigm is that the pituitary gonadotropins LH and FSH drive follicle growth. However, follicles can begin growth in the absence of pituitary hormones, and the follicles may grow to an early antral state with barely detectable levels of gonadotropins. Clearly, granulosa and thecal factors, as well as factors secreted by the oocyte itself, can also influence follicle growth, adding layers of complexity with paracrine, autocrine, and intracrine levels of control. Recent studies targeted the intraovarian mechanisms that initiate the growth of primordial follicles into primary follicles and beyond, as well as the complex, interacting regulatory networks that integrate gonadotropins and ovarian factors. For example, recent studies showed that FSH, estrogen, and insulin-like growth factor (IGF) all converge on the forkhead family of transcription factors in the ovary, which, in turn, controls the transcription of many target genes to affect apoptosis and cell cycle progression, among other important processes.

Ovarian follicles do not develop beyond the primary stage without growth differentiation factor-9 (GDF-9). Immature female rats treated with GDF-9 had more primary and small preantral follicles, but fewer primordial follicles, which suggests that GDF-9 recruits primordial follicles for maturation. GDF-9 did not affect later stages of follicle development. In contrast, FSH increased the number of small and large preantral follicles, but did not influence the number of primary or primordial follicles, which suggests that FSH acts mainly on the development of more advanced follicles. Thus, it seems that there are two distinct stages of follicle maturation, with the early stage dependent on input from the oocyte, and the later stage dominated by gonadotropin action.

The granulosa cells and thecal cells also express factors that are important in follicle maturation. Müllerian inhibitory substance (MIS) and the MIS type II receptor are expressed in the granulosa and thecal cells of both preantral follicles and small antral follicles, but not in later follicles. These results are intriguing because they suggest that MIS, best known for its role in promoting the apoptosis of Müllerian ducts to allow normal male embryonic development, may also have a role in the ovarian function of adult females. In preantral follicle culture, MIS enhanced both basal and FSH-stimulated growth, as measured by follicle diameter and cell number, but did not affect differentiation. Perhaps surprisingly, given its role in male development, MIS did not promote apoptosis in the follicle culture system. In contrast to MIS, another transforming growth factor (TGF), TGF- β , was a potent proapoptotic factor for preantral follicles in culture. These results suggest that MIS acts in the selection of dominant follicles; because MIS-expressing follicles are larger when they differentiate, they may be better able to produce estrogen and angiogenic factors and may have a competitive edge over other follicles in the cohort. This hypothesis, in turn, suggests that dominant follicle selection begins earlier than the antral stage.

OOGENESIS, OVULATION, AND LUTEINIZATION

Oogenesis is the process of oocyte cytoplasmic maturation and meiosis that produces mature eggs ready for fertilization by sperm. Immature oocytes communicate with their surrounding

follicular cells to coordinate the maturation of both the oocytes and the somatic follicle. Follicle maturation culminates in the rupture of the dominant follicle and the release of its oocyte. Ovulation, a complex process involving tissue remodeling and local inflammatory reactions, triggers the resumption of meiosis in the oocyte to produce a fertilizable egg. Meanwhile, the ruptured follicle luteinizes, or remodels itself into a corpus luteum, the main role of which is to help the uterus establish and maintain pregnancy until the placenta is ready to take over these functions.

The interplay between oocytes and granulosa cells

The mammalian oocyte and its companion granulosa cells are interdependent partners in follicle development. The important end result of this coordination is the release of an oocyte that is ready for fertilization and embryogenesis. Researchers have scant knowledge of how oocytes form into developmentally competent, mature eggs, or of the nature of the signals between granulosa cells and oocytes in developing follicles. In one approach to identify the potentially important gene families and proteins involved in folliculogenesis and oogenesis, researchers used mouse germ-cell specific transcript libraries and subtractive hybridization screens. Such screens identified several novel germ-cell specific transcripts, including *Oosp-1* (oocyte secreted protein) and *Nobox* (newborn ovary homeobox-encoding gene), that are candidates for further molecular and functional studies.

GDF-9, an oocyte-derived growth factor, affects the function of the surrounding somatic follicular cells. Related factors, the bone morphogenetic proteins (BMPs) and their receptors are present in rat ovaries. Insight into the roles of GDF-9 and the BMPs in reproduction have recently emerged through work conducted by the SCCPRR centers at the University of California at San Diego, Stanford, and Baylor. The data revealed that GDF-9 and the BMPs act in a complex pathway of ovarian regulation. Scientists from the SCCPRR center at Baylor generated mice lacking either one, or both alleles of the BMP-15 gene and bred them with mice lacking either one, or both alleles of the GDF-9 gene. The researchers determined that the dosage of intact BMP-15 and GDF-9 alleles directly influenced the extent of ovarian abnormalities. Interestingly, the BMP-15 gene has been linked to fertility regulation in Inverdale sheep; it is also a candidate gene for POF in women.

Other investigators are using transplant-based approaches to study these topics. When oocytes from secondary follicles were transplanted into primordial follicles, the primordial follicles doubled their rate of development, including the rate of differentiation of the somatic follicle cells. Importantly, the transplanted oocytes were normal and fully amenable to fertilization and embryonic development. Researchers concluded that oocytes possess certain inherent properties that control the rate of development of their surrounding follicle.

Regulation of oocyte meiosis

In vertebrates, oocytes begin development during embryonic stages, and then arrest at birth during the first stage of meiosis (meiosis I). During this arrest, the oocyte nuclear membrane (called the germinal vesicle) is intact and maturation promoting factor (MPF), an important regulator of meiosis, is inactive. Just prior to ovulation, MPF activity increases, the germinal vesicle breaks down, and the oocyte completes meiosis I. MPF activity is, in turn, controlled by phosphatases that regulate the progression of the cell cycle in meiosis and mitosis. Mutant male

mice that were unable to synthesize Cdc25b, a key phosphatase, were fertile; but the mutant females were sterile because their oocytes were permanently arrested at meiosis I. However, mutant oocytes that were microinjected with Cdc25b mRNA resumed meiosis, proving that the Cdc25b protein is a necessary and sufficient trigger for MPF activation. This study offered the first genetic model for studying the mechanisms that regulate prophase arrest in any vertebrate. This model provides new ideas about possible causes of certain types of female infertility in humans.

Novel mode of female contraception

The development of new contraceptives is prominent in efforts to control world population growth. The most desirable contraceptives would prevent the union of sperm and egg, cause minimal disruption of reproductive cyclicity, and exhibit minimal side effects. One promising target is interference with egg maturation or meiosis. Scientists at the SCCPRR center at Stanford University, in collaboration with the pharmaceutical company Organon, reported that a phosphodiesterase-3 (PDE-3) inhibitor prevented the maturation of oocytes, without disturbing the normal processes of follicular development, sex steroid secretion, ovulation, and reproductive cyclicity in rodents. Based on this work, a CRI involving Organon, the Oregon National Primate Research Center, and the SCCPRR center at Stanford University is now conducting proof-of-principal studies for this contraceptive approach in non-human primates. Results have demonstrated that PDE-3 inhibitors are active in blocking maturation of primate oocytes in culture, an effect that is reversible upon removal of the inhibitor. Current studies are evaluating the efficacy of these inhibitors *in vivo*.

Ovulation

Although the most prominent role of progesterone is maintaining a pregnancy, it is also essential for ovulation. An analysis of progesterone receptor knockout (PRKO) mice demonstrated that they lack two protein-degrading enzymes necessary for ovulation. In normal mice, transcription of the proteases ADAMST-1 and cathepsin L was induced at ovulation; but these proteases were not expressed in PRKO mice. As progesterone-responsive genes that are transcriptionally activated in granulosa cells at the time of ovulation, ADAMST-1 and cathepsin L represent the first clues about what cell types and proteases are important for ovulation. This work also showed that MMP-2 and MMP-9, matrix metalloproteinases (MMPs) thought to be involved in matrix remodeling prior to ovulation, are not targets of progesterone receptors. Thus MMPs may act independently of progesterone to promote ovulation.

Follicular atresia

Gonadotropins control follicular development by determining, in a given cycle, which of the growing follicles will continue to develop, and which will become atretic. Follicular atresia is thought to occur in the absence of necessary growth factors and/or in the presence of cytotoxic signals. Recent studies have identified a variety of factors that influence granulosa cell apoptosis. The Fas antigen (Fas) is a cell-surface receptor that induces apoptosis when bound to Fas ligand. Granulosa cells isolated *before* the LH surge were susceptible to apoptosis induced by Fas ligand or serum withdrawal, while cells isolated *after* the LH surge were resistant to apoptosis. In addition, theca cells were sensitive to Fas-mediated apoptosis before and after exposure to the LH surge. Such work suggests that granulosa cells from preovulatory follicles

become resistant to apoptosis after the LH surge, and that this resistance may be important for normal ovulation and luteinization.

Researchers are also studying factors that promote or inhibit granulosa cell apoptosis using normal granulosa cells and a cell culture system of spontaneously immortalized granulosa cells. Basic fibroblast growth factor (bFGF) prevents the calcium influx seen in granulosa cells destined for apoptosis; a molecule that inactivates calcium is just as effective as bFGF at preventing apoptosis. Newer studies showed that bFGF maintains calcium homeostasis by activating a protein kinase pathway that removes calcium from the cell. Researchers concluded that the ability of bFGF to regulate intracellular calcium concentration is the key to its anti-apoptotic activity.

Scientists are also studying the role of the cell adhesion molecule, E-cadherin, in preventing granulosa cell apoptosis. Cells cultured with an E-cadherin antibody became apoptotic. In these cells, Akt kinase activity was stifled, and caspase-3, the “executioner” molecule, was free to trigger apoptosis. Therefore, E-cadherin binding is an important factor in granulosa cell viability. A granulosa cell protein may act as a low-affinity, but high-capacity membrane receptor for progesterone; this membrane receptor may explain progesterone inhibition of granulosa cell apoptosis, despite the lack of the classic, nuclear progesterone receptor.

Remodeling of the tissues of the female reproductive tract

The extracellular matrix provides the physical support for ovarian cells; interactions between the cells and the matrix are critical for cell viability, as well as for normal steroidogenesis and ovulation. Remodeling of the ovarian extracellular matrix accommodates the growth of the ovarian follicle, the rupture at ovulation, and the transformation of the ruptured follicle into a corpus luteum.

The hormone relaxin is important for the growth and remodeling of reproductive and other tissues during pregnancy and parturition. Although relaxin was one of the first reproductive hormones to be identified, and its binding sites are widely distributed, the receptor for relaxin has remained a mystery. In an exciting breakthrough in January 2002, researchers showed that the leucine-rich, repeat-containing, G protein-coupled receptors LGR7 and LGR8 are receptors for relaxin; they mediate relaxin’s effects through a cAMP-dependent pathway. In addition, treatment of pregnant mice with the soluble ligand-binding region of LGR7, which was expected to act as a relaxin antagonist, delayed birth. The near ubiquitous distribution of the two relaxin receptors suggests that, in addition to its role in reproduction, relaxin may have important effects in many other organ systems.

UTERINE PHYSIOLOGY

The inner surface of the uterus is lined by the endometrium, the thickness of which changes cyclically as estrogen levels fluctuate. Endometrial tissue is highly specialized for the sole purpose of establishing and maintaining pregnancy. After ovulation, rising progesterone levels promote differentiation of the endometrial stroma, to prepare the uterus for implantation. If fertilization occurs, the resulting blastocyst, with the aid of lytic substances secreted by the

uterus, penetrates the endometrial cells during implantation. Elevated progesterone levels, mainly produced by the ovarian corpus luteum, are crucial to maintaining pregnancy. If there is no pregnancy, the corpus luteum declines, and the endometrium is sloughed off, which results in menstruation.

Decidualization and implantation

The decidual cells secrete substances that act on the ovary and uterus, causing the changes that are essential for successful pregnancy. Growth and differentiation of the decidua are the earliest adaptations to pregnancy by the uterus. Beyond the fact that it is essential for maintaining pregnancy, little is known about how progesterone acts on the uterus. In one study of this topic, researchers identified genes with temporal and spatial expression patterns that were consistent with mediating progesterone's effects on the uterus. Under the influence of progesterone, the endometrial stroma proliferated on day three of pregnancy in the mouse. Members of the "Hedgehog" family of signaling pathway genes, expressed in the luminal epithelium and glands and in the underlying mesenchymal stroma, were strongly up-regulated in the uterus on days three and four of pregnancy in mice. Other results demonstrated that progesterone (but not estrogen), acting primarily through progesterone receptors, induced the expression of hedgehog genes in the uterus of ovariectomized mice. The localization and regulation of hedgehog gene expression strongly support the hypothesis that progesterone's effects on implantation are indirectly mediated through hedgehog factors.

The blastocyst must burrow through the endometrium and embed itself within the stroma for successful implantation. The endometrial tissue must allow for this invasion, while maintaining its structural integrity. Tightly regulated stromal secretion of proteases, known as MMPs, helps to degrade the endometrium. Studies revealed a previously unrecognized interrelationship between progesterone and an inflammatory cytokine, interleukin-1 α (IL-1 α), in the regulation of MMP-3 in the tissue. IL-1 α stimulates MMP-3 in cultured, proliferative-phase endometrium. Progesterone blocks the stimulation of MMP-3, both *in vitro* and *in vivo*, by reducing the sensitivity of the stroma to IL-1 α . By decreasing sensitivity to IL-1 α , progesterone may ensure the overall structural integrity of the endometrium and developing placenta, despite the local disruption necessary for implantation and placentation. A better understanding of how the endometrium regulates MMP expression during pregnancy could contribute to improved treatments for infertility, placental defects, and pregnancy loss.

Gene profiling during the window of human implantation

A scholar in the Women's Reproductive Health Research (WRHR) program working in the SCCPRR center at Stanford provided an exciting new breakthrough with large-scale, micro-array profiling of genes expressed in human endometrium during the implantation window. Genes whose expression increased near the time of implantation included those involved in cholesterol transport, prostaglandin action, and immune function, among others; genes down-regulated at the time of implantation included those involved in G-protein signaling, calcium signaling, extracellular matrix, and cell adhesion, among others. The results of this study open new directions in basic, applied, and clinical research in implantation biology and will assist in the diagnosis and detection of implantation-related disorders.

Molecular mechanism of menstruation

During each menstrual cycle, the uterine endometrium proliferates in preparation for pregnancy. If implantation does not occur, progesterone levels decline, and the superficial layer of the endometrium sloughs off, resulting in menstrual bleeding. Normal menstruation is an important issue for women's quality of life and reproductive health. Important studies have demonstrated a potential link between vascular endothelial growth factor receptor-2 (VEGFR-2) and the MMPs, enzymes that degrade the extracellular matrix. In humans and monkeys, VEGFR-2 is specifically and dramatically up-regulated in the stromal cells in the premenstrual phase; expression of MMPs also follows this pattern. Because VEGF can enhance MMP expression, researchers hypothesize that VEGF acts through the VEGFR-2 to increase MMP expression in the superficial zone of the primate endometrium during the premenstrual phase to induce menstruation.

REPRODUCTIVE MEDICINE AND GYNECOLOGY

Pelvic floor dysfunction

Studies in the under-researched area of pelvic floor disorders and their sequelae will provide much needed information on the anatomy and physiology of dysfunctional states, such as pelvic organ prolapse, urinary incontinence, and anal incontinence, which affect women's long-term reproductive health and quality of life. The Branch's RFA on pelvic floor disorders has funded the development of methods and models to evaluate normal and abnormal pelvic floor anatomy and function. A high-resolution magnetic resonance imaging technique was established to evaluate the anatomy that supports the pelvic floor. Further, rhesus macaques were established as a primate model of pelvic organ prolapse because the basic pelvic floor anatomy is similar in female rhesus macaques and women; and, in macaques as in women, the pelvic connective tissue is responsive to estrogen.

Endometriosis

Endometriosis affects up to 50 percent of reproductive-age women and is the primary cause of infertility in approximately 6 percent of infertile couples. Endometriosis is a chronic disease manifested by pelvic pain and infertility; it is defined as the presence of endometrial glands and stroma outside of the uterus. Scientists recently recognized that the inflammatory reaction might contribute to the pathophysiology of endometriosis. Endometriotic lesions secrete chemokines, small chemical attractants that draw immune cells into the abdominal cavity. As the immune cells accumulate, they may cause the inflammatory symptoms and pain associated with endometriosis. Previous studies demonstrated that the specific chemokine RANTES is synthesized by endometriotic stromal cells and circulates in peritoneal fluid, at levels correlated with the severity of endometriosis. Newer studies showed that human peritoneal macrophages are capable of responding to circulating RANTES, and that the migration of immune cells is proportional to the RANTES concentrations in peritoneal fluid, and to the severity of endometriosis. Interfering with RANTES synthesis, or the subsequent influx of inflammatory cells into endometriotic lesions, be important targets for combating infertility and pain associated with endometriosis.

Many women with endometriosis are infertile; although there are many hypotheses, the exact cause of their infertility remains unclear. Ovulatory dysfunction, such as inadequate ovarian steroid secretion or compromised luteal function, is a potential cause for infertility associated with endometriosis. This model is strengthened by the observation that even women with mild endometriosis have signs of impaired granulosa cell function. Recently, scientists reported results that support this ovulatory dysfunction hypothesis. Using glycoprotein inhibin B as a marker of granulosa cell function, researchers compared patterns of inhibin B secretion in women with endometriosis-associated infertility, and in women with tubal-factor infertility during ovulation induction. Throughout stimulation, serum levels of inhibin B were significantly lower in women with endometriosis, than in women with tubal factor infertility. Researchers retrieved fewer oocytes from women with endometriosis and noted that these women secreted less estrogen, which suggests that endometriosis may impede the recruitment or maturation of follicles.

Polycystic Ovary Syndrome (PCOS)

PCOS is the most common cause of infertility in reproductive-age women. Abnormalities experienced by women with PCOS include ovarian cysts, irregular menstrual cycles, infertility, insulin resistance, and increased androgen production. Increased frequency and amplitude of LH pulses are common in women with PCOS, although wide variations in the prevalence of elevated LH levels are reported. Earlier research suggested that, in PCOS, the GnRH pulse generator is less sensitive to negative feedback by ovarian steroids. Blockade of androgen action with flutamide may restore the sensitivity of the GnRH pulse generator to estrogen and progesterone. Therefore, reduction of androgen secretion or blockade of androgen action may be important elements in restoring normal ovarian regulation of GnRH secretion in PCOS. This finding may have implications for treatments aimed at establishing cyclic ovulation in women with PCOS.

Anovulation is also an important cause of infertility, and many women with PCOS fail to ovulate spontaneously. Further, ovulation induction is particularly challenging in patients with PCOS. A retrospective study determined hormonal, metabolic, and ovarian morphological characteristics that predict an ovulatory response to pulsatile GnRH therapy in patients with PCOS. Successful ovulation in response to pulsatile GnRH was associated with higher baseline FSH levels, a blunted androgen response to hCGs, and lower body mass index and fasting insulin levels. Many women with PCOS are insulin-resistant and have compensatory hyperinsulinemia, which appears to contribute to chronic anovulation. Administering the insulin-sensitizing agent, metformin, to women with PCOS profoundly increased the incidence of ovulation in clomiphene-treated women, who were previously resistant to clomiphene therapy for inducing ovulation. Together, these studies demonstrated that pulsatile GnRH or metformin therapy were viable options for ovulation induction in certain patients with PCOS who are unresponsive to clomiphene citrate and to other standard therapies used to stimulate ovulation.

In addition to difficulty conceiving, women with PCOS are at increased risk of miscarriage (30 percent to 50 percent) during their first trimester. A retrospective study showed that women with PCOS who received metformin throughout pregnancy had significantly fewer miscarriages than untreated women with PCOS (8.8 percent and 41.9 percent losses, respectively). None of the children born to the metformin-treated mothers showed any adverse effects. Previous studies already established that insulin-sensitizing drugs are beneficial for preventing hyperinsulinemia,

improving ovulation, and decreasing serum testosterone in women with PCOS. This more recent study supports the hypothesis that decreasing hyperinsulinemic insulin resistance with metformin decreases the rate of early pregnancy loss, as well.

The incidence of PCOS in the general population of women is 4 percent, but cases of PCOS seem to cluster within families, which suggests that PCOS is a genetically inherited disorder. A five- to six-fold increase in the incidence of PCOS was found among first-degree female relatives of affected patients, compared with the prevalence of PCOS in the general population. Until molecular markers can be identified, a positive family history appears to be the most informative risk factor for the development of PCOS.

MALE REPRODUCTION

The male reproductive system produces sperm, the germ cells that carry a man's genetic complement to the next generation. It is a dynamic, inter-connected system of endocrine and support organs. The testes are responsible for both sex steroid production, and sperm production in the adult. The adult testes consist of germ cells, in all stages of development, as well as the support, or somatic cells. The somatic Leydig and Sertoli cells respond to gonadotropins (LH and FSH) that emanate from the pituitary gland. At puberty, FSH surges cause Sertoli cells to multiply greatly, and subsequently, the Sertoli cells to regulate sperm production (spermatogenesis). Leydig cells respond to LH by synthesizing and secreting testosterone. Testosterone is critical for optimal spermatogenesis and the maintenance of the male sex accessory glands, as well as for maintaining normal sexual function.

ENDOCRINOLOGY OF THE TESTES

Several lines of investigation have established the importance of LH and FSH signaling in normal testicular function. Male mice with mutations in the receptor for LH, found exclusively on Leydig cells, are infertile due to underdeveloped genitalia and sex accessory organs and disrupted spermatogenesis. Testosterone replacement therapy markedly improves this phenotype, which suggests that most of the effects of LH are mediated directly by the androgens that are produced in response to LH. The mechanisms of FSH action, however, are less clear. Men and mice with mutations in either FSH or its receptor on Sertoli cells are infertile due to lack of sperm production (azoospermia). This situation suggests that FSH stimulates the secretion of factors from the Sertoli cells that are critical for the progression of spermatogenesis. Further, FSH acts through its receptor to stimulate the production of the molecule cAMP. Presumably cAMP, directly or indirectly, turns on the FSH-responsive genes that are critical for spermatogenesis. The protein CREB, a direct target of cAMP, also stimulates the expression of certain genes. The presence of a mutant of CREB that is unresponsive to cAMP, when transfected into Sertoli cells *in vivo*, did not affect the Sertoli cells; but it caused the sperm to die within days, which supports the idea that FSH can act through CREB to exert its downstream effects on spermatogenesis.

Studies of FSH receptors (FSHRs) have provided additional information on the mechanism of FSH action. The number and activity of FSHRs determines the effects of FSH stimulation, so it is important to understand how the FSHR gene is controlled. Studies have shown that the FSHR gene is likely regulated through alterations in the chromatin structure that surround the coding region of the gene. The expression of the FSHR is dictated, in part, by FSH itself—the ligand acts to decrease the expression of the FSHR. This decrease in expression follows changes in chromatin structure from an “open” state, which permits gene transcription, to a “closed” or repressed state. Histone deacetylases and DNA methyltransferases mediate the change to a repressed state, although the pathway from FSH binding to these molecules has yet to be elucidated.

MIS is another hormone that is important in testicular function. Produced by fetal Sertoli cells, MIS was discovered and named for its role in inducing regression of the female reproductive tract structures (Müllerian ducts) in developing males. However, MIS persists at high levels through pre-pubertal development. Two laboratories have shown that MIS inhibits Leydig cell proliferation and testosterone production, events that herald the onset of puberty. A single injection of MIS significantly decreased testosterone levels in rats and mice and inhibited the expression of enzymes necessary to produce sex steroids. This work showed that, in addition to its role in embryonic development, MIS also regulates Leydig cell function.

Previous studies of rats showed that the level of androgens in the blood is not predictive of the level in the testes, where steroid hormones are present at much higher concentrations. In rats, levels of intratesticular testosterone can decrease drastically with no apparent effect on spermatogenesis. Although similar studies were not feasible in humans because available techniques were too invasive, now a minimally invasive method allows researchers to assess the intratesticular fluid in humans by needle aspiration. Using this method, researchers found that the concentration of testosterone within the testes is about 100 times greater than the level in the blood. More importantly, the development of this procedure will allow further studies of the human intratesticular environment and its correlation with normal spermatogenesis.

SPERMATOGENESIS

Chromatin packaging

In addition to producing the hormones necessary for normal sexual development and function, the testes also produce spermatozoa. In the past several years, powerful genetic approaches have elucidated the mechanisms of spermatogenesis. For example, research revealed that the dramatic compaction of the DNA in sperm is dependent on the exchange of general DNA-packaging proteins, called somatic histones, with sperm-specific proteins, called transition proteins (TPs), and protamines. By eliminating either TP-1 or TP-2, researchers produced male mice with severely reduced fertility and abnormal DNA packaging. In these mice, histones were normally displaced, possibly through the up-regulation of the remaining TP; protamines also bound normally. However, protamine 2 (Prm2) accumulated abnormally, and chromatin was not completely condensed, possibly increasing susceptibility to DNA strand breaks. These results suggest that, while spermatogenesis can proceed in the absence of one type of transition protein, fertility is optimal when both TP-1 and TP-2 are present.

Mice and humans have two different protamines, Prm1 and Prm2, a situation that is somewhat unusual among mammals. Studies showed that decreasing the gene dosage of either protamine disrupted nuclear structure and processing of Prm2, and reduced sperm motility in mice. Thus, both protamines must be present in normal amounts for proper sperm function and fertility. Protamine transcripts are made in round spermatids, but are not translated until the elongating spermatid stage. Premature translation of Prm1 mRNA causes early nuclear condensation, which blocks spermatogenesis at the round spermatid stage. Investigators have shown that a highly conserved sequence in the 3' untranslated region of Prm1 mRNA is solely responsible for regulating this translation. Mutations in the protamines and TPs did not affect sperm production, per se, suggesting that defects in these proteins may be responsible for some cases of idiopathic male infertility in which sperm production appears normal.

The molecular mechanisms of how TPs and the protamines replace the somatic histones remain unclear; however, studies have provided an unexpected insight. The enzyme, Ca²⁺-calmodulin dependent protein kinase 4 (Camk4), is highly expressed in spermatids; in other systems, the Camks regulate gene transcription, the “reading” of the DNA into mRNA. Male *Camk4* knockout mice were infertile, and their sperm development was arrested at the elongating spermatid stage. The arrested sperm had abnormal Prm2 expression, as well as nuclear abnormalities that were reminiscent of the protamine knockouts. Because Prm2 can serve as a substrate for Camk4, it seems likely that Camk4 is a part of the molecular pathway that replaces TPs with protamines during the final stages of spermatogenesis.

Meiosis and spermiogenesis

Before DNA can be packaged into sperm by TPs and protamines, germ cells must undergo meiosis. Investigators discovered a protein critical for this process in sperm. Cyclin A1 is expressed in mammalian germ cells, and its absence causes male sterility. In mice with mutant cyclin A1, spermatogenesis stops at the first meiotic cell division, and the developing germ cells undergo apoptosis. Interestingly, females without cyclin A1 exhibit no defects in oogenesis, which demonstrates that the mechanisms of meiosis are different between males and females.

Researchers have also described mutations that affect the post-meiotic development of germ cells, in addition to the defects described above. Spermiogenesis is the process by which round spermatids mature into spermatozoa, the fully mature sperm capable of swimming. Absence of the transcription regulator Trf-2 in frogs and worms results in embryonic lethality, but, surprisingly, mice lacking Trf-2 are viable. However, these male mice have small testes and are sterile because the developing sperm fail to progress from the round spermatid stage to the elongating spermatid stage. Coincident with this failure, researchers found reduced expression of post-meiotic genes, including those that encode the TPs and the protamines. Thus, it appears that Trf-2 may regulate differentiation by selectively activating specific target genes in round spermatids.

Interactions between germ cells and Sertoli cells

The mutations described thus far apply to genes specifically expressed in germ cells. Analogous to the interdependence of oocytes and somatic follicular cells in females, scientists have long hypothesized that the somatic Sertoli cells are intimately involved in spermatogenesis. Work on

the Sertoli cell factor *Dax-1* has provided strong support for this hypothesis. *Dax-1* is a nuclear receptor best known for its role as a transcription factor during normal adrenal and gonadal development. Mice that lack *Dax-1* showed degeneration of the seminiferous epithelium that was independent of pituitary and testicular hormones; this phenotype does not occur in men with inactivating *DAX* mutations. Further, these knockout mice were infertile and showed Leydig cell hyperplasia. When *Dax-1* was replaced in Sertoli cells, fertility was restored, although some testicular and sperm abnormalities remained. This latter finding suggested that full testicular function requires *Dax-1* in other somatic cell lineages, perhaps in the Leydig cells. The identification of the downstream targets of *Dax-1* will further elucidate the mechanisms whereby Sertoli cells regulate sperm development.

Dax-1 represses transcription of steroidogenic factor-1, indicating yet another role for this factor in steroidogenesis. The *Dax-1* mutant mice showed significantly greater expression of the gene that encodes aromatase, the enzyme that converts testosterone to estradiol, and higher levels of intratesticular estradiol. Tamoxifen, an estrogen inhibitor, restored fertility and partially corrected the Leydig cell hyperplasia. In men with *DAX-1* mutations, the phenotype of adrenal hypoplasia and hypogonadotropic hypogonadism may result from estrogen excess caused by an overabundance of aromatase. Together, these studies showed that *Dax-1* has three distinct roles in the testes: influencing testis structural development, regulating spermatogenesis, and regulating steroidogenesis.

In addition, scientists have focused much of their efforts on genes on the male-specific Y chromosome that presumably control male fertility. However, *DAX-1* is one example of an X-linked gene that is important to male fertility. A recent study indicated that *DAX-1* is not an unusual case and suggested that the X and Y chromosomes may be equally important in spermatogenesis. A systematic search for genes expressed only in spermatogonia (the self-renewing, mitotic germ cells) identified 25 genes, 10 of which were X-linked. This finding challenges the paradigm that the Y chromosome evolved as a “degenerate” X chromosome, retaining or co-opting genes necessary for spermatogenesis, and suggests that the X chromosome makes an unexpectedly large contribution to male fertility.

SPERMATOGONIAL STEM CELLS

Sperm develop from spermatogonial stem cells in the testes. These stem cells continuously renew themselves, reproducing by mitosis throughout almost the entire lifetime of human males. The stem cells can also enter a differentiation pathway that leads to the formation of mature sperm. Identifying signals that promote self-renewal versus differentiation is critical to understanding stem cell fate and of spermatogenesis.

In male *Drosophila* (fruit flies), the germline stem cells attach to a group of somatic support cells called “the hub.” A recently discovered ligand called Unpaired is uniquely expressed in the hub; Unpaired activates the JAK-STAT signaling pathway. This finding is striking because, in the absence of JAK-STAT signaling, all cells adopt the differentiated fate, while ectopic expression of JAK-STAT promotes self-renewal of the germline stem cells. This result suggests that Unpaired is secreted from the hub and acts through JAK-STAT to promote self-renewal of the

germline stem cells. Interestingly, JAK-STAT signaling is also required for the maintenance of mammalian embryonic stem cells, which suggests that genetic studies of *Drosophila* germ-line stem cells hold great promise for revealing the mechanisms governing stem cell fate in mammalian systems.

One of the most significant advances in the past several years has been the development and refinement of spermatogonial stem-cell transplantation techniques. In this powerful technique, spermatogonial stem cells proliferate *in vitro*, allowing the genetic manipulation of the cells followed by transplantation into recipient testes. Investigators used this process to produce transgenic mice and transplanted these mice's spermatogonial stem cells to restore fertility in mutant recipient mice that lacked their own germ cells. The feasibility of this technique in larger mammals was demonstrated with the successful transplantation of germ cells into pigs. These studies raise the prospect of restoring spermatogenesis in human males who have lost spermatogonial stem cells due to radiation or chemical insults, as is the case with many cancer patients, or those that harbor genetic defects precluding normal sperm production.

Investigators have also taken advantage of this technique to explore basic questions in sperm biology. For example, they found that the time required for sperm to fully mature is unique to each mammal; the time span was generally considered to depend on the intimate relationship between the spermatogenic cells and the Sertoli cells within the seminiferous tubules. Investigators transplanted rat germ cells into mouse recipients and observed that spermatogenesis proceeded with the timing and stages characteristic of the rat, not the host mouse. As with oocytes, the data indicated that spermatogenic progression is pre-programmed by the germ cell, and not dependent on the somatic compartment of the testes. These data have implications for cell-cycle control in stem cells, and for the possibility of utilizing cross-species transplantation to circumvent certain causes of infertility.

In another study, germ cells from *tfm* mice, which lack functional androgen receptors, were transplanted into recipients with normal somatic cell androgen receptors. The transplanted cells completed meiosis in the normal recipients. This study demonstrated that germ cells, per se, do not require androgen receptors to progress through spermatogenesis and, therefore, the direct action of androgens occurs through the somatic cells of the testes.

SPERM MOTILITY

Nearly all known organisms use the molecule cAMP as an intracellular signal to transmit instructions received from the cell surface to the cell nucleus, and to influence gene expression. A class of enzymes called cyclases produces cAMP. Classical cyclases span the cell membrane, with one end outside the cell and one end inside the cell. Recently, researchers described a new "soluble" class of cyclases. Soluble cyclase (sAC) responds to different modulators and is part of a different signaling pathway than classical cyclases. Because sAC is uniquely abundant in male germ cells, it could be important in producing functional sperm. Scientists demonstrated that bicarbonate stimulates sAC, a fact that delineates a mechanism for the bicarbonate-induced cAMP increase in sperm which ultimately results in sperm motility, capacitation, and the

acrosome reaction. This discovery could be of broad biological significance because sAC is widely expressed in other bicarbonate-sensitive tissues.

Scientists have hypothesized that cAMP modulates sperm motility through stimulation of the enzyme protein kinase A (PKA). PKA consists of regulatory subunits (RI or RII subunits) and catalytic subunits (C subunits). RII subunits help localize the C subunits to certain regions of the cell membrane, presumably to those areas closer to the substrates of the C subunit. One study used mutant mice that lacked the RII subunit to investigate the pathway of PKA-stimulated sperm motility. Surprisingly, the resultant mice were fertile. Therefore, the RII-dependent localization of the C subunit is not necessary for sperm motility. Mice that lacked functional C subunits suffered from severe growth retardation; the animals that survived to adulthood produced morphologically normal, but immotile sperm, which demonstrates the absolute requirement for active PKA in sperm motility.

However, PKA is not the only regulator of sperm motility. A newly discovered molecule, Catsper2, is present on the sperm flagellum and is presumably involved in regulating the entry of calcium into the sperm cell. Catsper2 is very similar to a previously discovered sperm calcium channel, Catsper. Absence of Catsper results in male sterility due to immotile sperm. Given its flagellar localization, it is also likely that Catsper2 is involved in the regulation of sperm motility. Although calcium is a critical determinant in cell behavior in other systems, these data together represent the first reports that calcium is also essential for sperm motility. Since both the Catsper and Catsper2 calcium channels are sperm-specific, it may be possible to design contraceptives to specifically disrupt sperm flagellar channels without affecting other crucial calcium channels in the body.

MALE REPRODUCTIVE TRACT

Sperm cells produced by the testes need the active support and contributions of the male reproductive tract, particularly the epididymis, to complete their maturation into fully functional cells capable of fertilization. The mechanisms by which this occurs are unclear; however, investigations of estrogen receptor gene knockout (ERKO) mice have provided some insight into the function of the male reproductive tract. These mice are unable to respond to estrogen and, as expected, the females were sterile. Surprisingly, ERKO males were also infertile. The ducts leading from the testis to the epididymis became swollen with excess unresorbed fluid, causing testicular atrophy and the cessation of sperm production. A detailed investigation of ERKO males revealed a more widespread occurrence of abnormalities, including: aberrant growth of the efferent ductules; abnormal specialized cells in specific epididymal regions; and the accumulation of glycogen, a carbohydrate not normally stored in the male reproductive tract. This research also showed that estrogen was critical in maintaining the epithelial differentiation of the efferent ducts and regulating ion transport across this epithelium.

REPRODUCTIVE TECHNOLOGY AND CONTRACEPTION

With the burgeoning use of the mouse as a genetic model for human disease, it is imperative to have an efficient and economical method to store the germ cells of potentially useful mouse mutants. A new method of freeze-drying mouse sperm maintains the genetic integrity of the stored sperm, which can produce live offspring when used with intra-cytoplasmic sperm injection (ICSI), a process that involves the direct injection of a sperm into an egg. This simplified methodology is not only useful to scientists studying mice, but also to those interested in preserving sperm from valuable animals of many mammalian species.

Male contraceptive development also remains a priority to the RSB. Interference with the blood-testis barrier provides an intriguing contraceptive lead. The blood-testis barrier, formed by tight junctions between neighboring Sertoli cells, prevents the passage of molecules from the blood into the seminiferous tubules. However, this barrier does allow for the passage of developing germ cells. Research showed that a synthetic peptide derived from the molecule occludin, which helps to form the tight junctional complex, reversibly disrupted the blood-testis barrier in mice. Administration of this peptide caused male sterility, but, after the peptide was withdrawn, the testes of treated males were repopulated by the spermatogonial stem cells, and males regained their fertility. Thus, interference with the dynamic process of Sertoli cell tight junction assembly may provide a new target for male contraceptive development.

REPRODUCTIVE MEDICINE AND ANDROLOGY

Scientists have defined three distinct regions of the Y chromosome that are critical for normal spermatogenesis: Azoospermic Factor (AZF) a, b, and c; further, they have identified genes within these regions and have begun to characterize the regions' normal function. Such efforts are a critical first step in helping men with "idiopathic" azoospermia, 30 percent of whom have microdeletions of the Y chromosome.

Data from one study indicated that the majority of human male AZFa deletions are caused by homologous recombination between two endogenous retroviral elements that flank the AZFa locus. In addition to explaining the molecular mechanism behind the AZFa deletion that causes male infertility, this study was also the first report of provirus-provirus recombination disrupting a human gene, which established provirus sites as possible disease mutation hotspots.

A similar process may cause deletions in AZFc. Investigators recently sequenced the entire AZFc region (4.5 megabases), which had been stubbornly resistant to sequencing. The results demonstrated that AZFc is a complex of six distinct families of massive repeat units, which does not contain any single-copy sequences. This research not only provides a resource for identifying all AZFc transcription units as candidate genes for spermatogenesis defects, but also explains the high *de novo* mutation rate of AZFc in male infertility and allows insight into the evolution of the Y chromosome.

The most common molecular cause of spermatogenic failure in men is deletion of the AZFc region, which contains the *DAZ* (deleted in azoospermia) gene family. *DAZ*-encoded RNA-

binding proteins are present in the nuclei and cytoplasm of fetal gonocytes, and in the nuclei of spermatogonia. The DAZ proteins translocate to the cytoplasm during meiosis. An autosomally encoded form of the protein, called DAZL, persists in mature sperm, which suggests that the DAZ gene family is important at multiple stages of spermatogenesis, from the establishment of the spermatogonial stem cell pool through meiosis. Research recently identified a member of the DAZ gene family, called *BOULE*, on chromosome 2, that is considered to be the evolutionary precursor to *DAZL*, which, in turn could be the ancestor of the Y chromosome *DAZ*. Deletion of the *Drosophila* (fruit fly) homolog *Boule* caused meiotic arrest and infertility. Phylogenetic analysis suggested that *Boule* is the ancestral gene, that *DAZL* arose in vertebrates, and that *DAZ* is only found on the Y chromosome of primates. The evidence suggested that the primary role of *DAZL/DAZ* is in early germ cell development and function, while *BOULE* is more important for regulation of meiosis.

The precise function of *DAZ*, an RNA-binding protein with unknown RNA targets, was delineated using a technique named SNAAP, or specific nucleic acids assoiated with proteins. Several mRNAs that bind to *DAZ* were isolated, including the mRNA that encodes Tpx-1, a testicular cell adhesion protein that is necessary for spermatogenesis. Identification of these *DAZ*-bound RNAs will allow new studies of exactly how the *DAZ* deletion results in infertility.

DEVELOPMENTAL BIOLOGY OF REPRODUCTION

Sperm, egg cells, and all the cells that are capable of developing into sperm and egg cells are referred to as the germline. The net result of a healthy adult reproductive system is the maintenance of the germline, both in an individual, and into the next generation. Through the process of fertilization, eggs and sperm join to begin the formation of a new organism. From the time of fertilization, to early cleavage, and through the formation of the blastocyst, the new organism is referred to as the preimplantation embryo.

The blastocyst contains all of the cells that are needed to form the embryo, its germ cells, and later, the fetus and the placenta. The inner cell mass of the blastocyst develops into the embryo and fetus and is also the source of embryonic stem cells. In mammals, the continued development of the embryo and fetus are absolutely dependent on implantation, the process in which the blastocyst embeds in the uterus. The trophoblast, or outer wall of the blastocyst, is primarily responsible for implantation; subsequently it develops into the placenta.

During embryo development, primordial germ cells (PGCs), the undifferentiated progenitors of male and female gametes, migrate into the genital ridge area; this process induces differentiation of the genital ridge into the sex-appropriate fetal gonads and accessory reproductive organs. Through this process of sex determination, the generative cycle begins all over again.

THE GERMLINE

Germ cell fate

One of the key mysteries of embryo development is how the germline cells, those that eventually develop into sperm and eggs, are established and maintained separately from the somatic cells that form all of the other parts of an organism. Some of the most useful insights into this process of the determination of germ cell fate come from studies of organisms such as fruit flies (*Drosophila*) and worms (*C. elegans*). A critical and defining quality of both somatic stem cells and germline stem cells is their ability to self-renew, as well as to produce large numbers of differentiated progeny. Scientists discovered that a gene called *piwi* is required for the asymmetric division of germline stem cells in *Drosophila*, a process by which the totipotent germline stem cell divides into a daughter stem cell and a differentiated daughter cell. The identification of the *piwi* gene thus represents a very important gain in the understanding of germline stem cell renewal.

In worms (*C. elegans*), the PIE-1 protein is an essential regulator of germ cell fate that localizes to the germ cell lineage during the first cleavages of the embryo. One function of PIE-1 is to inhibit mRNA transcription, which, in turn, blocks the potential germ cell (PGC) from entering the somatic cell lineage. Scientists have now shown that PIE-1 has a second function in germ cells, one that is genetically separable from the transcription effect: PIE-1 is required for efficient expression of a maternally encoded gene called Nanos homolog (*nos-2*). *Nos-2* is essential for primordial germ cell development. A mutation in the second zinc finger domain of PIE-1 reduces *nos-2* expression without affecting transcriptional repression; this mutation also causes PGCs to move away from the somatic gonad, occasionally exiting the embryo entirely. Thus, PIE-1 promotes germ cell fate by two independent mechanisms: (1) inhibition of transcription, which blocks the zygotic programs that drive somatic development; and (2) activation of protein expression from *nos-2* and possibly other maternal RNAs, which promote PGC development. The study of germline development has clear relevance to human health because the germline is essential for reproduction and the perpetuation of the species. Abnormal germ cell development can lead to severe developmental abnormalities in humans, including the formation of gonadal tumors. In a more general sense, understanding how germline establishes and maintains properties of totipotency and immortality may provide novel insights into methods for tissue regeneration and the treatment of infertility.

Induction of primordial germ cells in mammals

In many species, PGCs are specified by maternal factors in the egg cytoplasm; in mammals, however, evidence suggests that specification of PGCs occurs later in development, during gastrulation. Results from transplantation experiments suggest that factors produced by the extraembryonic ectoderm (EEC) are required for PGC formation because transplanted epiblast cells are most likely to develop into PGCs when placed in close proximity to the EEC. Two BMPs, Bmp4 and Bmp8, are localized to the same cells in the EEC, and both are required for PGC generation, which makes them candidate factors for PGC generation. Recent studies showed that these growth factors were both required for induction of PGC formation. After inactivating both growth factors in various experiments, investigators proposed that Bmp4 was required in initial steps of PGC specification, as early as the blastocyst stage in the inner cell mass. Subsequently, the sensitized cells responded to the combined action of Bmp4 and Bmp8

and developed into PGCs. Future studies will determine the receptor actions and signaling pathways involved in PGC determination in mammals.

Despite recent advances in the ability to manipulate spermatogonial stem cells, knowledge of the molecular basis for germline development during mammalian embryogenesis is poor. Scientists used a novel combination of established molecular and transgenic approaches in a systematic molecular-genetic study of mammalian germline development. They established cDNA libraries from PGCs at discrete stages of development of transgenic animals. These cDNA libraries will allow researchers to identify and characterize genes that are only expressed in primordial germ cells at each separate stage. Using these findings, they can determine the function of each gene in germline development to greatly improve the understanding of germline cell development.

TRANSGENESIS

The use of transgenesis has revolutionized biomedical science, allowing scientists to make specific genetic alterations that can be transmitted to future generations through the germline. For more than 20 years, transgenesis has made it possible to produce genetically altered mice as models of human genetic diseases, and to provide a deeper understanding of how a fertilized egg can develop into all of the cells of the body. Transgenesis is accomplished by either the injection of a foreign gene into an egg cell nucleus, or by fusing genetically altered embryonic stem cells with formative, inner cell mass (ICM) cells of a mouse blastocyst. The resulting offspring carry the foreign gene as part of their normal chromosomal constitution. Studies of transgenic mice have yielded large amounts of information about human genetic diseases and disorders. However, for certain diseases, as well as for vaccine development, a primate model would be advantageous. Scientists have accomplished a first step toward this model in monkeys. One group of researchers injected a modified virus that was connected to the desired test gene into monkey eggs and fertilized the eggs using ICSI. The developing embryos were transferred to a surrogate female monkey. The offspring were then tested to be sure that the transgene was faithfully transmitted to the somatic cells of many organs. Out of 20 transfer procedures, one transgenic male monkey was born. When he reaches sexual maturity in about three years, researchers will test his fertility. Scientists now need to determine which human diseases they will study first using this type of procedure, a debate that raises numerous additional technical and ethical questions that must be addressed prior to the start of future experiments.

Efforts to produce a single transgenic monkey demonstrated that traditional means of transgenesis are efficient only for mice, and that alternative methods are highly desirable. To that end, researchers found a more amenable ICSI-based system of transgenesis. Mouse sperm heads that were incubated with pieces of DNA retained that DNA; when this complex was injected into egg cytoplasm by ICSI, the resulting progeny mice carried the test DNA as part of their normal chromosomal constitution. Unlike other methods, transgenesis by ICSI may be easily adaptable for use in other mammalian species, including monkeys. This technique may allow the full use of transgenesis in model systems most relevant to human disease, which has the potential to elicit new methods of treatment and prevention.

FERTILIZATION AND ACTIVATION OF DEVELOPMENT

Fertilization is a complex process of cell-cell interactions between egg and sperm; the sperm must reach the egg within a narrow window of time, penetrate a set of accessory cells and a tough barrier membrane surrounding the egg, and bind to and fuse with the cell membrane of the egg to enter the egg cytoplasm. At some point in this process, the sperm must pass a species-specificity test. Within seconds of a sperm's penetration of the egg, a cascade of reactions are initiated in the egg that constitutes the activation of development.

Intracytoplasmic sperm injection (ICSI)

ICSI, the direct injection of a sperm into an oocyte, allows infertile men whose sperm are incapable of penetrating the egg, to father children. ICSI is now commonly practiced in human fertility clinics around the world. But, because the procedure lacks a solid basis in animal experimentation, there is much concern about the possible long-term effects on offspring generated through this procedure, including the possibility that children conceived by ICSI may inherit infertility, as well as the presence of indications of sex chromosome abnormalities and other possible anomalies in these children.

Using rhesus monkeys conceived by ICSI, scientists are assessing the short- and long-term effects of ICSI on animals whose physiology and development closely resembles those of humans. Thus far, the data from these ICSI monkeys has indicated that sperm undergo abnormal processing when injected into the oocyte through the ICSI procedure. This processing could form the basis for sex chromosome anomalies that may occur when ICSI is used in humans. Longitudinal studies that follow human offspring conceived by ICSI are necessary to assess potential long-term effects.

Development of polarity in mammalian eggs

Morphological and molecular polarity of eggs is readily apparent in lower organisms. Until very recently, however, this molecular polarity was not clear in the mature oocytes of mammals. Morphological polarity results from the existence of an amicrovillar region overlying the meiotic spindle and the microvillar region to which sperm attach. This asymmetry develops during oocyte maturation, when the symmetrical, germinal vesicle-intact oocyte, with its centrally located nucleus, transforms into the asymmetrical mature oocyte with an off-center meiotic spindle and asymmetric distribution of components of the plasma membrane and egg cortex. Studies have shown that the acquisition of morphological polarity, manifested by the amicrovillar and microvillar zones, as well as by the location of nuclear material, is accompanied by the redistribution of molecules that are involved in sperm-egg interactions. Drugs that perturbed actin microfilaments or microtubules also altered the asymmetric distributions of sperm-egg binding sites and often perturbed the localization of chromosomes. Thus, these studies confirmed and extended the findings of earlier studies that showed polarity in the plasma membrane and cortex of mammalian eggs involved molecular remodeling and was determined by the location of nuclear material.

Egg activation

Following fertilization, one of the first events in egg activation is a significant increase in intracellular calcium. This increased calcium level is required for continued egg activation and

for the subsequent steps of development in all species studied, including humans. Injection of sperm or sperm extracts stimulates activation of eggs, suggesting that a sperm factor is involved in the calcium increase. Recent work on sea urchins showed that high concentrations of nitric oxide synthase were present in activated sperm. Furthermore, sea urchin eggs produced nitric oxide within seconds of insemination, preceding the calcium pulse. Injection of nitric oxide donors into eggs mimicked the sperm-induced calcium pulse; injection of oxyhemoglobin, a nitric oxide scavenger, blocked egg activation by sperm. The evidence showed that nitric oxide-related bioactivity occurred in the proper intracellular sites, and at appropriate times, to stimulate egg activation. Furthermore, the study showed that egg activation could not occur in the absence of nitric oxide. At least in sea urchin eggs, nitric oxide is both necessary and sufficient for successful fertilization. Studies are underway to determine if nitric oxide plays the same role in mammalian fertilization.

First crystal structure for protein involved in sperm-egg fusion

Despite more than a century of strong interest in understanding sperm-egg interactions during fertilization, the reproductive sciences community still has much to learn. Animals such as sea urchins, frogs, and abalone are useful in this quest because they produce huge numbers of eggs, allowing the researchers to isolate sufficient amounts of proteins and carbohydrates for direct biochemical isolation and characterization of the interacting molecules involved in fertilization. Thus, these organisms may offer clues into the nature and types of interactions that may be involved in fertilization in mammals.

Surprising new work has shown that the genes for male and female interacting proteins may be closely linked in the abalone genome, which suggests co-evolution of genes that are important for fertilization. Investigators have isolated a chromosome segment from abalone sperm that contained the gene for VERL (Vitelline Envelope Receptor for Lysin), a portion of the sperm lysin gene itself, and a newly characterized sperm acrosomal gene called *sp18*. The proximity of these genes provides interesting insights into the evolution of molecules that are important for fertilization. Further sequence analysis of *sp18* and the closely linked lysin genes showed that they are derived from a single, ancient gene that duplicated and then diverged rapidly through evolution. While the lysin protein recognizes the vitelline envelope in a species-specific way and dissolves a hole in it, *sp18* fuses with the plasma membrane surface of the egg cell itself. The two closely linked genes provide the sequential control of the species-specific interaction between the sperm and the vitelline envelope (or zona pellucida in mammals), as well as the interaction between sperm cell membrane and egg cell membrane. This work is highly important because it could provide new insights into certain types of infertility in humans; or it may allow development of novel contraceptive approaches.

In addition, scientists recently solved the crystal structure of *sp18*. This is the first crystal structure obtained for a molecule involved in fusion between the cell membranes of sperm and egg. This type of research may provide the only system for detailed study of the key interacting molecules in animal fertilization. The conservative nature of fertilization throughout evolution suggests that understanding human fertilization will be greatly improved by such studies.

PREIMPLANTATION DEVELOPMENT

The preimplantation embryo, as the immediate product of fertilization, is the earliest phase of human embryo development and is critical for implantation and establishment of pregnancy. As such, the preimplantation embryo plays the key role in the clinical practice of human *in vitro* fertilization, as well as in preimplantation genetic diagnosis (PGD), a method of detecting genetic diseases and disorders prior to the establishment of pregnancy.

Preimplantation development in culture

Mammalian embryos develop inside the mother's oviduct and uterus; therefore, without the formulation of preimplantation embryo culture media, knowledge of early mammalian development would be virtually nonexistent. The first 24 hours of *in vitro* mammalian development is one of the most sensitive periods because it encompasses the first cleavage. To learn more about this time period, one study compared the early development of hamster embryos in the oviduct versus those in culture. Calcium homeostasis, which is critical to cleavage in mammals and non-mammals alike, was a key outcome measure. After only six hours of *in vitro* culture, one-celled hamster embryos had significantly elevated intracellular calcium levels, as compared with embryos newly isolated from the oviduct. In addition, mitochondria in the cultured embryos were abnormally distributed, lactate metabolism was greatly decreased, and the *in vitro* embryos were less able to develop into blastocysts than the comparably staged embryos from the oviduct. When the intracellular calcium concentration was reduced, the abnormalities were eliminated, and development to the morula and blastocyst stages improved. The ability to control calcium homeostasis is an excellent example of how knowledge of the cell physiology of early embryos can be used to improve culture media.

Mouse embryos cultured in a special medium, called KSOM/AA, can develop to the blastocyst stage at rates that are close to the rate of blastocyst formation *in vivo*. Furthermore, based upon physiological, morphological, and molecular criteria, blastocysts raised in KSOM/AA are of higher quality than those raised in inferior media. An important report showed that blastocysts cultured in KSOM/AA maintained the correct genomic imprint for the *H19* gene; in contrast, blastocysts raised in Whitten's medium, an inferior medium that has been widely used for years, lose imprinting of some genes, such as *H19*, but not others, resulting in aberrant gene expression patterns. Thus, culture conditions can dramatically, but selectively, affect the expression of imprinted genes. This research is an outstanding demonstration of the importance of improving media for human preimplantation embryo culture, an issue of increasing clinical relevance. Increasingly, more fertility clinics are relying on blastocyst culture for PGD and the selection of a single, healthy blastocyst to transfer into a woman's uterus, thus avoiding high-risk pregnancies with multiple fetuses.

Insulin and preimplantation development

It is well documented that women with PCOS have increased rates of miscarriage, as well as elevated levels of insulin-like growth factor (IGF-1) and insulin. Preimplantation embryos raised *in vivo* and *in vitro* that are exposed to high concentrations of IGF-1 or insulin are significantly less likely to form blastocysts and are more likely to form degenerate embryos. A high concentration of IGF-1 or insulin causes damage to the IGF-1 receptor that, in turn, causes extensive apoptosis of the inner cell mass (ICM) of the mouse blastocyst; the ICM differentiates

and develops into the entire fetus. Additional research supported and elaborated on these earlier findings. In one study, scientists induced high IGF-1 concentrations in embryo culture media and *in vivo* using IGF-1 pellets implanted in the mouse uterus. Both sets of experiments showed that high concentrations of IGF-1 during preimplantation development *in vitro* or *in vivo* led to increased resorption of the embryos. The clinical implications of this research may be profound in that elevated ambient concentrations of IGF-1 may be responsible for the high prevalence of early embryo loss in patients with PCOS. Thus, altering the IGF-1 and insulin concentrations in women with PCOS, prior to conception and/or in the preimplantation period, may help to reduce the likelihood of miscarriage in these women.

Rate of preimplantation development

The timing of the transition from zygote to blastocyst is an important developmental parameter. Earlier cleavage of both hamster and human embryos is correlated with an increased likelihood of the blastocysts' development and survival to birth. The gene that regulates the rate of development in mouse embryos is named *Ped* (preimplantation embryonic development). The *Ped* gene has two alternative forms, *Ped fast* and *Ped slow*. *Ped fast* mice reach the early blastocyst stage in 89 hours, with about 32 cells, at which time the *Ped slow* animals are still at the morula stage, with only about 19 cells. In addition, *Ped fast* mice are larger as adults and have larger litters. The *Ped* gene encodes a major histocompatibility complex (MHC) protein called Qa-2. Recent work showed that two genes, *Q7* and *Q9*, control the production of the Qa-2 protein. When microinjected into the male pronucleus of *Ped slow* embryos, *Q7* and *Q9* change the slow-developing embryos into fast-developing embryos. These embryos display the Qa-2 protein on their surfaces, while the uninjected, slow embryos do not. These studies raise the interesting question of how MHC proteins function in early development, as opposed to their other, better known roles in cellular immunity. Researchers anticipate that these findings will have practical applications in farm animals and in humans.

Aneuploidy in early cleavage embryos influenced by modifier genes

About 30 percent of the spare human preimplantation embryos from *in vitro* fertilization clinics show chromosome mosaicism, a condition in which different cells of the early embryo have different numbers of chromosomes. This abnormal chromosome constitution most likely results from errors in chromosome segregation during mitosis. It is not known whether this condition results from some aspect of the *in vitro* procedures, or whether it is inherent in the egg or sperm. Aneuploidy (abnormal chromosome segregation) is usually fatal; however, embryos that do survive have two (or more) genetically different types of cells in their body. Investigators developed a mouse model of early cleavage stage aneuploidy to study the influences of *in vitro* procedures and to establish the role of genetic background in chromosome segregation during embryo cleavage. These mice carry a Y chromosome that is prone to malsegregation, so that aneuploid progeny carrying either an extra Y chromosome, or no Y chromosome, are common. These studies indicate that the Y chromosome is stably transmitted during meiosis, but is lost by non-disjunction during mitosis shortly after fertilization. The genetic background influences the susceptibility for Y chromosome non-disjunction, which offers a strong argument that modifying genes have an influence on the non-disjunction process. This mouse model can provide new insights into the mechanisms involved in non-disjunction in humans and may explain the high rate of mosaicism found in human embryos from *in vitro* fertilization clinics.

Nuclear reprogramming

The recent excitement over animal cloning has led to many novel attempts to improve the success rate of somatic cell nuclear transfer. A related and equally important concern is understanding how the egg cytoplasm reprograms the transferred nuclei of somatic cells from its highly differentiated state to a totipotent state that will support embryo development. Studies are focusing on the molecular mechanisms involved in nuclear reprogramming of transferred nuclei. The first analysis of global changes that occur in the mRNA expression of bovine fetal fibroblast nuclei after transfer to enucleated bovine oocytes demonstrated that, by the time of blastocyst formation, the pattern of gene expression was indistinguishable between normal blastocysts (*in vitro* and *in vivo*) and those produced by nuclear transplant. Thus, the genes of the somatic cell nucleus were reprogrammed to behave as if they were embryonic genes, within just seven days.

Other studies evaluated the mRNAs for three key metabolic enzymes at the one-cell, two-cell, six- to eight-cell morula, and blastocyst stages in bovine nuclear transfer embryos and in control embryos produced by *in vitro* fertilization. By the blastocyst stage, the mRNA levels were comparable between nuclear transfer embryos and control embryos, and the mRNA expression patterns in both types of embryos differed from the expression pattern in the original fetal fibroblast cells. These results clearly indicated that factors present in the recipient oocyte cytoplasm were capable of reprogramming the newly transferred nuclei. It is still unclear if abnormalities associated with cloning result from dysregulation of other specific genes, or from adverse effects on genomic imprinting. Future studies will provide more detailed information about how this reprogramming occurs in different types of differentiated adult cells, which may lead to the identification of the adult cells most amenable to cloning. This approach will also likely reveal how factors, such as the culture media, can influence the course of the reprogramming.

Gene activation and repression in preimplantation embryos

The first dramatic molecular event after fertilization of the mammalian egg is embryonic genome activation (EGA). EGA is characterized by a massive onset of new, embryo-controlled, gene activity, as the gene products provided by the egg are degraded. The types of genes that are activated during EGA seem indiscriminate and global, from well-known housekeeping genes, to genes with no match in any current gene database. EGA is thought to represent a key feature in the reprogramming of the nucleus that converts the highly differentiated egg nucleus into the totipotent nucleus of the early cleavage stage embryos. New evidence indicated that, superimposed on this apparently promiscuous EGA and nuclear reprogramming, was the formation of a transcriptionally repressive state during the two-cell stage. This repression appeared to be mediated by chromatin structure because it was reversed by histone hyperacetylation or by inhibiting the second round of DNA replication. Reversing repression blocked further development of the two-cell embryo. These results suggest that the sum of two opposing processes, activation and repression, provided the final pattern of gene expression necessary for progression of the two-cell embryo into a blastocyst.

SOMATIC CELL NUCLEAR TRANSFER AND EMBRYONIC STEM CELLS

After many attempts, scientists successfully created the first mammalian clone, Dolly the sheep; poor success rates have been the norm with other, large animals. Numerous scientists are trying to refine the procedure and to discover why there is such a high rate of failure in cloning embryos by somatic cell nuclear transfer into an enucleated egg. In 1999, investigators successfully cloned mice using the nuclei from adult mouse somatic cells. This research was extremely important because it proved that the cloning of a sheep was not a single event, and that the procedure could theoretically be accomplished in any mammal. In this experiment, the donor nuclei for oocyte injection came from uncultured cumulus cells, the somatic cells that surround and support oocytes during their development. The live-born young were used in subsequent rounds of cloning, so clones of clones were obtained. Still, the low success rate of cloning caused concerns that cloning may cause severe damage and indicated that both short-term developmental studies and longitudinal studies of long-term effects were necessary to evaluate the impact of cloning on the animals produced. The cloned mice provide a resource for such studies and enable detailed molecular genetic studies on the reprogramming of somatic cell genomes, genomic imprinting, embryonic genome activation, and the very basic aspects of cell differentiation.

Embryonic stem (ES) cells

The fertilized egg divides and develops into every highly specialized cell type necessary for the formation of a new individual. The only diploid cells to rival this enormous potential are the ES cells derived from the ICM of a blastocyst. The use of ES cells holds incredible promise for scientific advances. The year 2002 was an historic one, marked by the first federal funding of research on human ES cells; in addition, the first human ES cell project was funded by the NICHD as an administrative supplement to a NICHD MERIT Award on *Egg-mediated motility during fertilization*.

ES cells can be used as donor cells, giving rise to genetically homogeneous animals critical to studying a broad range of important biological questions. In current animal cloning protocols, the nucleus of an ES cell is transferred to an enucleated mouse oocyte; this single ES cell nucleus can direct the full development of an egg into a live offspring. Previously, cloning by nuclear transfer was accomplished only with freshly isolated cells and with cells from primary cultures. Recently though, scientists cloned mice by transferring nuclei obtained from ES cells from a long-term cell culture that had been through many passages. This important advance provides a link between ES cell technology and animal cloning.

A companion study showed that clones derived from cultured ES cell nuclei survived post-implantation development significantly more often than clones derived from adult somatic cell nuclei. This research was the first to achieve the transfer of the nuclei of genetically altered mouse ES cells into enucleated oocytes to produce identical, genetically altered, live mice. Lines of genetically altered mice are valuable as models of human diseases and disorders, and for other functional genomics research. Genetically altered ES cells now provide an alternative to the cumbersome production of chimeric mice through blastocyst injection of engineered ES cells.

Using this technology, one potential method for therapeutic cloning would be transferring the nucleus of a patient's cell into an enucleated donor egg, developing that egg into a blastocyst, and deriving ES cells from it. The ES cells could then be coaxed into developing into a particular cell type that could be transplanted back into the patient. To date, two investigators have successfully implemented the first steps of this procedure in mice, starting with nuclei from fetal neuronal cells. The resulting ES cells resembled other types of ES cells, in that they had lost the neuronal markers, and could develop into all types of differentiated cells; that is, the neuronal cell nucleus was successfully reprogrammed to be totipotent, just like the natural nucleus of an egg cell. This experimental system can now be used to study the mechanisms of reprogramming, as well as to refine procedures for use in cell-based transplantation therapy.

Imprinting

Methylation in the upstream promoters of genes is an epigenetic modification that generally suppresses gene expression. Large portions of the mammalian genome are methylated on the cytosine residues of DNA, and certain genes show an "imprinted" pattern of methylation that is dependent on parental origin. For example, only the unmethylated copy of the *H19* allele inherited from the mother is expressed, while the heavily methylated copy inherited from the father is not. Because imprinting is parent-specific, methylation must be erased in primordial germ cells, and then re-established during gametogenesis, so that all sperm cells will bear a paternal imprint, and all oocytes will bear a maternal imprint. Normal mammalian development requires both male and female components in fertilized eggs.

Methylation patterns are established and maintained by DNA methyltransferases. The major methyltransferase, called Dnmt1, is not expressed in either mouse oocytes or preimplantation embryos; instead, they express an isoform called Dnmt1o. By creating knockout mice that lacked Dnmt1o, but expressed Dnmt1 as usual, investigators determined the role of Dnmt1o during oogenesis and preimplantation development. The knockout mice developed normally, but the females were infertile due to fetal death during late gestation. Methylation analysis of the oocytes from knockout females proved that the genomic imprints were established normally, but that methylation was lost unexpectedly from half of the normally methylated alleles of imprinted genes in the offspring, which caused fetal death. Dnmt1o was found in the nucleus only during the eight-cell stage, which led investigators to the hypothesis that Dnmt1o is required for maintenance of methylation during the 4th round of DNA replication. DNA replication is semi-conservative, with one strand acting as a template for a newly constructed strand. Therefore, without Dnmt1o to maintain methylation for this one round, half of the resulting double-stranded DNA is normally methylated, while the other half is not. In conclusion, these results suggest that, while Dnmt1o has a crucial role in very early development, another unknown methyltransferase is responsible for establishing *de novo* methylation in oocytes, and for maintaining methylation in preimplantation development.

SEX DETERMINATION

Each embryo begins as a sexually indifferent being that can develop into either a male or a female. Sex determination is the translation of the chromosomal sex (that is, XX, female, or XY, male) into the development of gender-appropriate internal and external reproductive structures. An improved understanding of how sex is determined in humans is important because errors in the process are fairly common and can range in severity from complete sex reversal (a person with a male XY genotype develops incorrectly as a female, or vice versa) to minor genital abnormalities. Sex determination, as an early embryological event, is a dynamic morphological process that can also help to address basic questions of gene expression, cell-fate determination, and hormone signaling. For example, several research highlights in this report demonstrated the remarkable versatility of the factors that act in sex determination, such as DAX-1 and MIS, as they continued to act in later development, and even mediated adult fertility.

Determination of phenotypic sex

The initial events of sex determination are dependent on the presence or absence of a normal Y chromosome. In the absence of the Y chromosome, the bipotential genital ridge develops into an ovary. The normal expression of the Y chromosome testis-determining gene, called *SRY*, initiates a cascade of gene interactions that ultimately results in testis development. Then the Leydig and Sertoli cells of the testes secrete factors, such as testosterone and MIS, which shape male secondary sexual characteristics. *SRY* is thus the long-sought “testis-determining factor,” and, in the normal course of events, it is the primary determinant of phenotypic sex. However, the downstream targets of *SRY* and thus the actual mechanisms that produce a male phenotype remain unknown.

A transgenic mutant mouse called Odsex is helping to answer questions about the relative role of *SRY* in sex determination. The Odsex mutation deletes a large region upstream of the *Sox9* gene, a testis-determining factor that is repressed in females. Deletion of this region in the Odsex mouse causes all affected XX mice to over-express *Sox9* in the gonad, and therefore to develop as phenotypically normal, but sterile, males. Thus, the Odsex mutation provides the first XX, *Sry*-negative, sex-reversed mouse. Because the sex reversal is strain-dependent, investigators used backcrosses in a genome-wide scan for loci that interact with the Odsex mutation. They found one major autosomal locus that was responsible for 93 percent of the variation in sex determination. These researchers are also generating Odsex mice that carry all Y chromosome genes except *Sry* to assess the role of Y chromosome genes in spermatogenesis, particularly to determine whether or not expression is required in the somatic cells of the testes.

Until recently, molecules that specifically affect testis formation, other than *SRY* and *SOX9*, though long sought, remained unknown. Scientists created a mouse that lacked the fibroblast growth factor 9 (*Fgf9*) gene to determine its role in early development. Unexpectedly, the knockout mice were predominantly female, and careful examination revealed that XY mice lacking *Fgf9* develop with a female phenotype that ranges from testicular hypoplasia to complete sex reversal. Further studies demonstrated that *Fgf9* acts in embryonic Sertoli cell development, proliferation of gonadal cells, and migration of cells from the mesonephros into the gonad. Thus, *Fgf9* is a mediator of many *Sry*-dependent events. However, unlike *Sry*, *Fgf9* homologs are

found in all vertebrates, as well as in other lower animals. Therefore, *Fgf9* may be the original sex determiner that, through evolution, has come under the control of *Sry*.

Development of the reproductive tract

Correct expression of *SRY* is the first step in a cascade of events that promote masculinization of the bipotential embryo. MIS, secreted by the Sertoli cells of the fetal testis, is another essential component. In males, MIS induces regression of the Müllerian ducts, the progenitors of the female internal reproductive tract, so that only the male (Wolffian) ducts remain. MIS induces its effects by binding to a heterodimer composed of a type I receptor and a type II receptor; the type II receptor for MIS is MISRII, but the type I receptor had long been unknown. Two groups of researchers simultaneously achieved the goal of determining the type I receptor for MIS, when they found that ALK2 is the MIS type I receptor. The MIS Type I receptor ALK2 is expressed in most tissues, so MIS signaling is primarily regulated by the presence of the type II receptor and the MIS ligand.

Cryptorchidism

During mammalian development, the testes descend from their original position in the abdomen, to their final home in the scrotum. Cryptorchidism, the failure of the testes to descend, is a frequent human birth defect that is associated with infertility and an increased risk of testicular cancer. Both hormonal and genetic factors affect the migration of the testes. Recently, a team of investigators identified a new mouse model of cryptorchidism. The mice have a transgenic insertion that causes cryptorchidism with complete sterility; the condition can be corrected through surgery to place the testes in the scrotum. The transgene that causes cryptorchidism in this mouse model was mapped to mouse chromosome 5, which contains the gene *Great*, a gene that encodes a novel G-protein coupled receptor. *Great* is expressed in the brain, in embryonic and adult gonads, and in the gubernaculum, a ligament that is key to testicular descent; but *Great* is not expressed in other organs. Mice lacking the *Great* gene had the same phenotype of high abdominal testes as the original transgenic mice, which proved that *Great* is responsible for normal testicular descent. The phenotype suggested that *Great* affects the first stage of testicular descent, which is androgen-independent.

The search for *GREAT* mutations in infertile men is now underway following the recent cloning of the human *GREAT* gene. Interestingly, *GREAT*, also called *LGR8*, was recently identified as the receptor for relaxin. Relaxin is mainly associated with remodeling of the female reproductive tract during pregnancy and parturition. Female *Great* knockout mice developed normally and were fertile, a fact which suggests that another, closely related LGR may have redundant functions in females. But, in males, *Lgr8* is specifically required for testes migration. Further, because INSL-3 is structurally similar to relaxin, and mutations of the *INSL-3* gene also cause cryptorchidism, *LGR8* may also be a receptor for INSL-3.

RESEARCH TRAINING AND CAREER DEVELOPMENT

TRAINING, FELLOWSHIPS, AND CAREER DEVELOPMENT AWARDS

The RSB supports the development of highly skilled reproductive science investigators through individual postdoctoral fellowships, institutional training grants, and career awards to funded scientists and clinicians. Individual postdoctoral fellowships (F32s) are awarded to newly trained young scientists for up to three years, which enables them to work full-time with a qualified mentor to develop expertise in an eligible field of reproductive science research. The RSB supported 31 F32 fellows during fiscal year 1999, 31 in fiscal year 2000, 35 in fiscal year 2001, and 28 in fiscal year 2002. Institutional training grants (T32s) are awarded to outstanding educators at leading institutions in the United States to establish and maintain an appropriate environment for reproductive sciences research training. Since 1999, the Branch has issued about 30 institutional research training grants each year, supporting approximately 70 pre-doctoral and 65 postdoctoral trainees selected by the recipient institutions. Last year, the RSB-supported training program at the University of Pennsylvania, directed by Dr. Jerome Strauss, was honored with the first NICHD Mentor award for outstanding institutional training programs.

The career award, or K series of grants, are made to scientists with outstanding research potential, so as to release that candidate from teaching and administrative responsibilities, and to allow a period of intensive focus on research. The Independent Scientist Award (K02) is used to promote the careers of newly independent scientists. The RSB supported four K02s in fiscal year 1999, five in fiscal year 2000, six in fiscal year 2001, and four in fiscal year 2002. In 1995, the NIH streamlined the career award series by instituting the Mentored Clinical Scientist Development Award (K08). Eight of these awards were active in the RSB during fiscal year 1999, six in 2000, six in 2001, and four in 2002. Two new mechanisms were announced in 1998, the Mentored Patient-Oriented Research Career Development Award (K23) and the Mid-career Investigator Award in Patient-Oriented Research (K24). The RSB supported one K24 grantee in fiscal year 1999, two in fiscal year 2000, five in fiscal year 2001, and six in fiscal year 2002. In 1999, the NIH instituted the Mentored Quantitative Research Career Development Award (K25) to encourage scientists with engineering or quantitative science backgrounds to pursue biomedical research. In 2000, the Branch made its first K25 award to a physicist who is studying DNA-protein interactions in sperm chromatin using optical trapping of single DNA molecules.

REPRODUCTIVE SCIENTIST DEVELOPMENT PROGRAM (RSDP)

The RSDP is a multidisciplinary, multi-institutional research career development program for obstetrician-gynecologists in cell and molecular biology and related fundamental sciences. The program is funded through a unique collaborative arrangement that includes support from the NICHD and several private organizations, such as foundations, professional societies, and pharmaceutical companies. This program uses the NIH Mentored Research Scientist Development Program Award (K12) mechanism. To date, the program has trained 47 reproductive scientists.

WOMEN'S REPRODUCTIVE HEALTH RESEARCH (WRHR) CAREER DEVELOPMENT CENTERS

The WRHR career development program was developed by the NICHD to support the careers of obstetrician-gynecologists who have recently completed postgraduate clinical training, and are beginning basic, translational, and/or clinical research in junior faculty positions. The ORWH and the NCI have joined the NICHD in supporting this program to increase the number of skilled obstetrician-gynecologist investigators through a mentored research experience that will lead to an independent scientific career in women's health. The program's emphasis is on research relevant to obstetrics and gynecology and/or its subspecialties: maternal-fetal medicine, gynecologic oncology, and reproductive endocrinology. Relevant fields, such as adolescent gynecology, urogynecology, and the reproductive health of women with disabilities are also included. Mentors with established research programs that cover a broad range of basic and applied biomedical and biobehavioral science in obstetrics and gynecology, together with collaborating departments, form a base for training WRHR scholars. The first RFA for the program was announced in 1998, and 12 institutions received awards that year. The RFA was reissued in 1999, and eight additional grants were awarded.

Currently, the Branch supports 20 WRHR centers, located at the following institutions:

- University of Colorado
- University of Pennsylvania School of Medicine
- Bowman Gray School of Medicine
- University of Cincinnati Medical Center
- Wayne State University
- Stanford University School of Medicine
- University of Texas Medical School at Galveston
- University of California, Los Angeles
- University of California, San Francisco
- University of Texas Medical School at Houston
- Oregon Health Sciences University
- University of Washington School of Medicine
- Brigham and Women's Hospital
- University of Utah Health Sciences Center
- University of Alabama at Birmingham
- University of California, San Diego
- Magee-Women's Hospital, University of Pittsburgh
- University of Rochester
- Case Western Reserve University
- Columbia University College of Physicians and Surgeons

Thus far, more than 60 scholars have been appointed to faculty positions in the 20 WRHR centers nationwide. These scholars represent a diverse group of investigators from various subspecialties and emerging areas of obstetrics and gynecology who are pursuing a broad range of basic science and clinical research topics.

BUILDING INTERDISCIPLINARY RESEARCH CAREERS IN WOMEN'S HEALTH (BIRCWH) CAREER DEVELOPMENT PROGRAMS

The ORWH developed the BIRCWH program as an institutional career development award. The BIRCWH program supports research career development of junior faculty members who have recently completed clinical training or postdoctoral fellowships, and who are commencing basic, translational, clinical, and/or health services research relevant to women's health. This

institutional award uses the NIH Mentored Research Scientist Development Program Award (K12) mechanism. The RFA was issued in 1999, and 12 grants were awarded in July 2000. Because of the success of the program, the RFA was reissued in 2002, and additional programs will join the program in the fall.

One goal of this initiative is to promote research and the transfer of findings that are relevant to women's health, including sex/gender similarities or differences in biology, health, or disease, through mentored research and a career development experience that leads to an independent, interdisciplinary, scientific career in women's health. The program bridges the gap between advanced training and research independence, as well as the gap between professions and scientific disciplines. The ORWH, along with nine NIH Institutes and the Agency for Healthcare Research and Quality (AHRQ), now support the 12 BIRCWH programs. The NIH Institutes and Centers support biomedical and behavioral research and research training; the AHRQ supports health services research and research training. The NICHD administers the program for ORWH and supports approximately 40 scholars at the following sites: Baylor College of Medicine, Washington University, University of Alabama, University of North Carolina, University of California at Los Angeles, University of Michigan, University of Connecticut Health Center, and University of Medicine and Dentistry of New Jersey.

FRONTIERS IN REPRODUCTION (FIR)

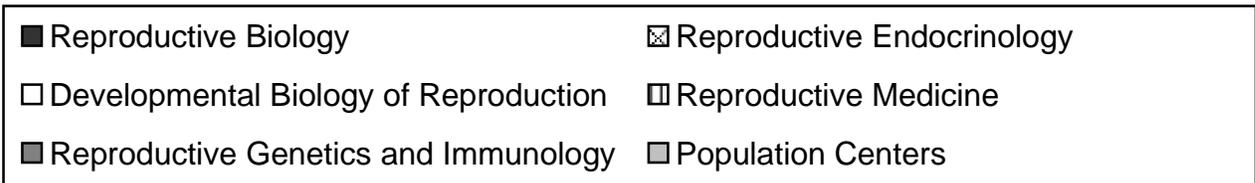
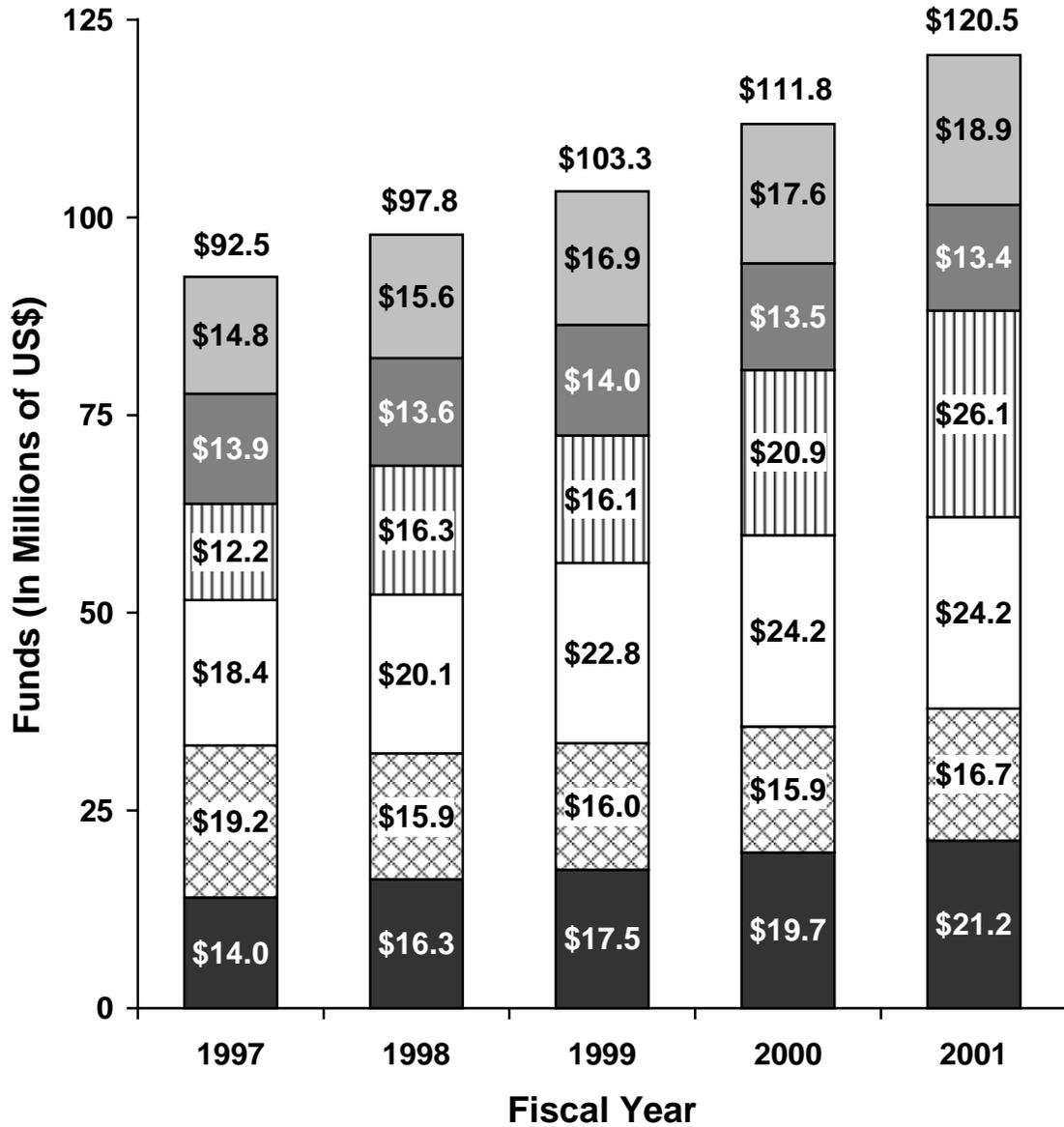
The RSB played a leading role in launching this international training course in techniques and concepts of advanced reproductive biology research. FIR debuted in 1998, at the Marine Biological Laboratory, Woods Hole, Massachusetts. Supported jointly by the NICHD and other professional and private sources, the course provides an intense, six-week learning experience to 16 postdoctoral or junior faculty scholars. A combination of laboratory work, lectures, and symposia brings together an international faculty and attracts applicants from as many as 23 countries. In 2001, the course and symposia were brought together under the multiyear, T15 training mechanism.

THE RSB SEMINAR SERIES

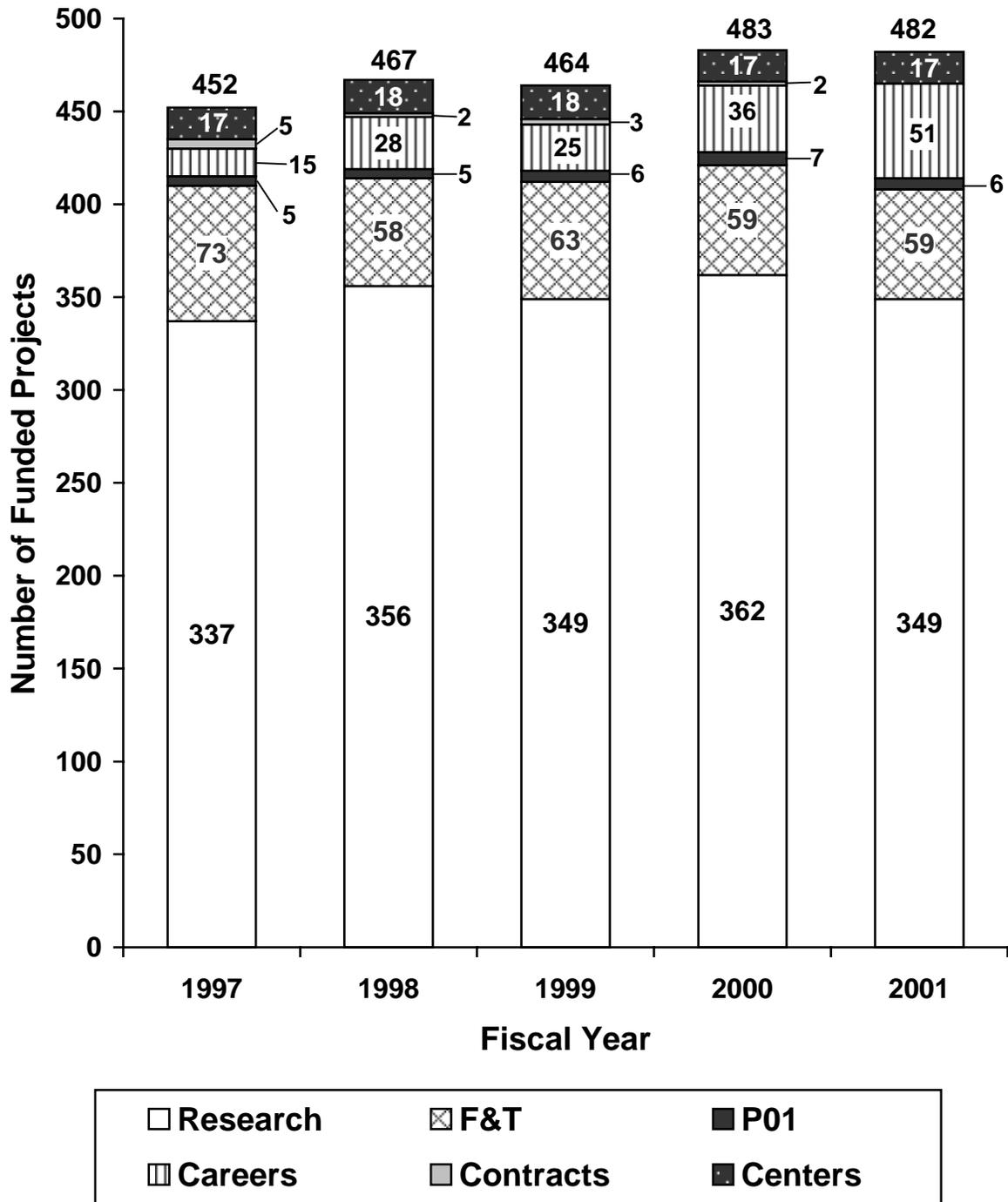
In 2001, the RSB instituted a Friday afternoon seminar series to disseminate exciting research findings among its grantees. Scientists who are just starting their careers and distinguished, established researchers alike are invited to present their latest research directions at an informal, hour-long seminar for the RSB and other interested professional staff. The seminars provide an excellent opportunity to exchange information between staff and grantees. The Branch plans to continue this successful program.

FIGURES

FIGURE 1: RSB PORTFOLIO BY RESEARCH CATEGORY—FUNDING:
FISCAL YEAR 1997-FISCAL YEAR 2001

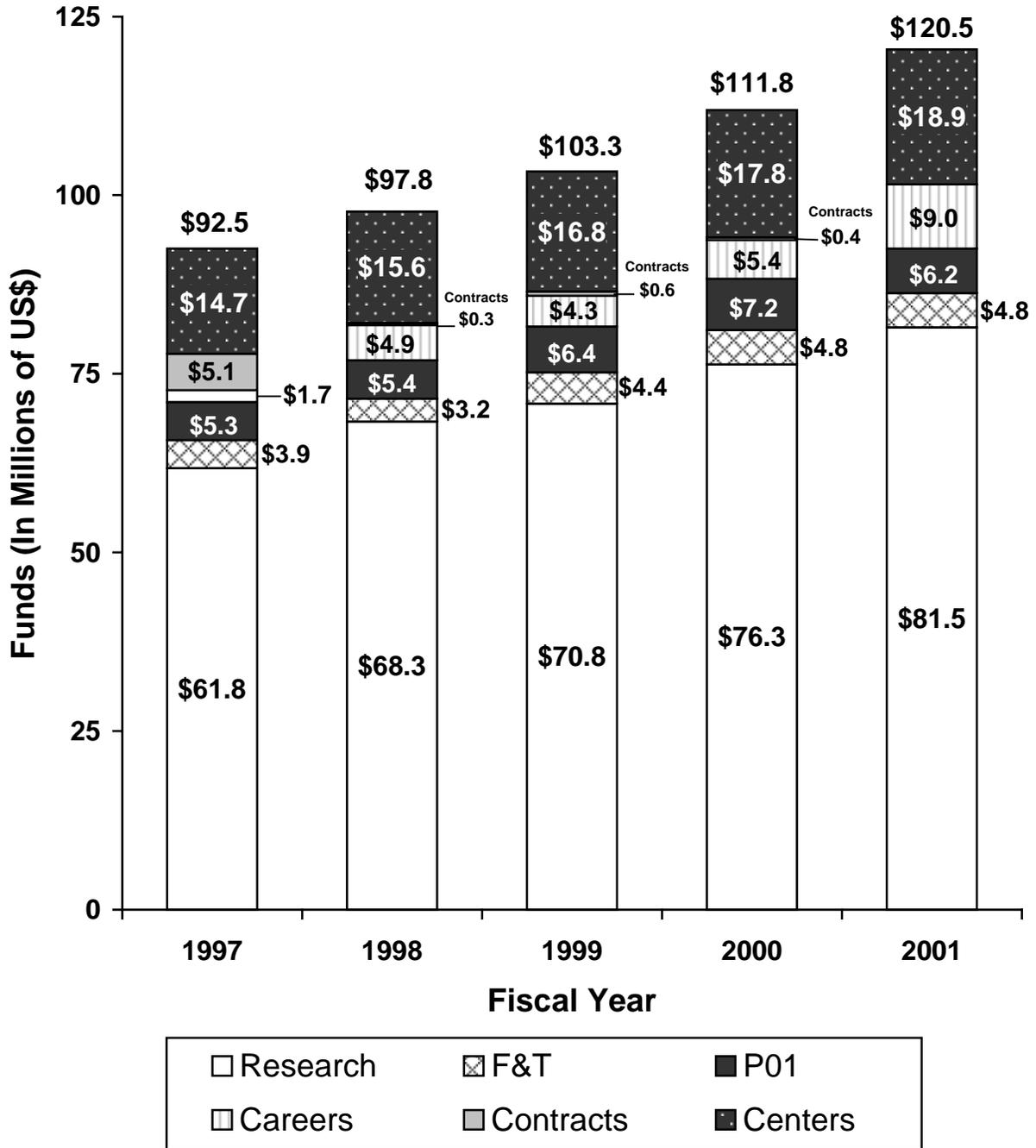


**FIGURE 2: RSB PORTFOLIO BY SUPPORT MECHANISM—NUMBER OF FUNDED PROJECTS:
FISCAL YEAR 1997-FISCAL YEAR 2001**



(Note: The RSB did not issue any contracts in fiscal year 2001.)

**FIGURE 3: RSB PORTFOLIO BY SUPPORT MECHANISM—FUNDING:
ISCAL YEAR 1997-FISCAL YEAR 2001**



(Note: The RSB did not support any contracts in fiscal year 2001.)

FIGURE 4: RSB PORTFOLIO BY RESEARCH CATEGORY, FISCAL YEAR 2001

**Total Funds in
Millions US\$: \$120.5**

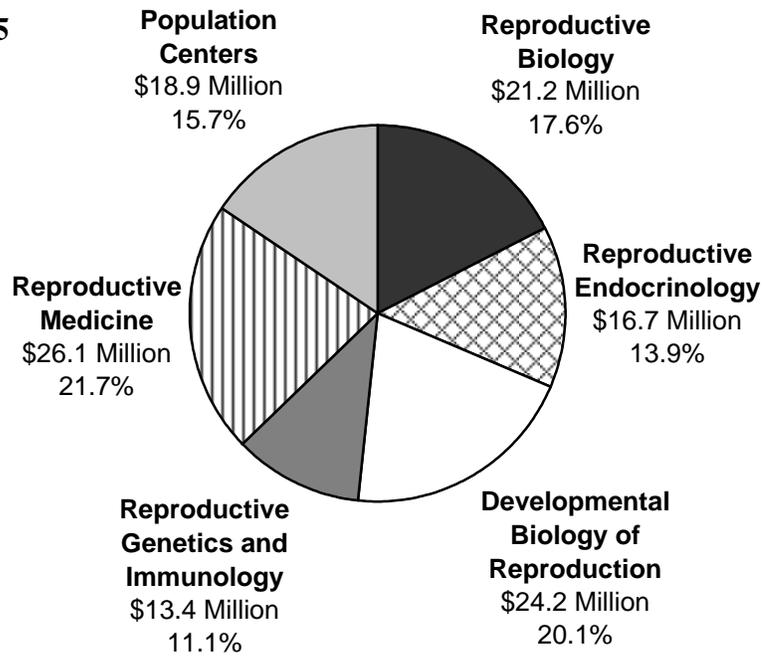
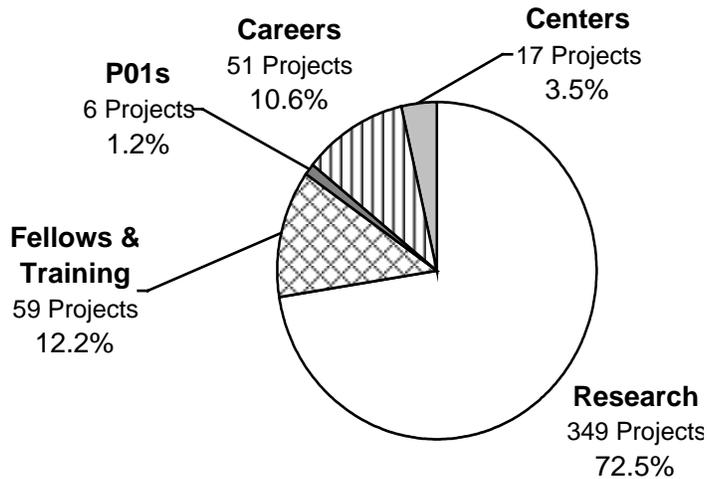


FIGURE 5: RSB PORTFOLIO BY SUPPORT MECHANISM, NUMBER OF FUNDED PROJECTS, FISCAL YEAR 2001

**Total Number of
Projects: 482**



(Note: The RSB did not issue any contracts in fiscal year 2001.)

APPENDIX A: RSB PERSONNEL

Phyllis C. Leppert, MD, PhD, came to the NIH in 1999, as the chief of the RSB, Center for Population Research, NICHD. She received her doctorate from Columbia University and her medical degree from Duke University. A board-certified obstetrician and gynecologist, Dr. Leppert was a professor and the chair of the Department of Gynecology and Obstetrics at the State University of New York at Buffalo prior to joining the federal government as RSB chief. She was also on the medical school faculty at the University of Rochester, where she was chief of the Obstetrics and Gynecology Department at Rochester General Hospital; prior to that position, she was a member of the faculty of Columbia University. Her academic research career has focused on remodeling the extracellular matrix of the uterus and cervix throughout gestation, and on the interaction of cells and matrix proteins in reproductive tissues. Throughout her career, she has also conducted clinical research in women's health and was a Robert Wood Johnson Clinical Scholar at Duke University. Dr. Leppert is the co-editor of *The Extracellular Matrix of the Uterus, Cervix and Fetal Membranes* and the senior editor of *Primary Care for Women*. She is currently the program officer of the SCCPRR and the NCPIR, as well as the research coordinator for the National Cooperative RMN. In addition, Dr. Leppert is a member of the program committee for the Society for Gynecologic Investigation, and a member of the Fellowship Committee for the American Gynecological and Obstetrical Society.

Joan C. Davis, MD, MPH, joined the RSB in the spring of 2002, from the Health Resources and Services Administration (HRSA), Bureau of Health Professions, at the Department of Health and Human Services (DHHS). She received her undergraduate degree in biology from Williams College, her medical degree from the State University of New York at Buffalo, School of Biomedical Sciences, and her master's in public health from the Johns Hopkins School of Public Health. She also did her residency at Johns Hopkins School of Medicine in general preventative medicine. Her previous activities include work as a clinician for the Bureau of Sexually Transmitted Disease, New York City Department of Health, and as a consultant physician in a domestic violence shelter in New York City. Dr. Davis also serves as the director of the BIRCWH program administered by the NICHD. Her portfolio encompasses basic, clinical, interdisciplinary, and translation research in women's health, including sex/gender similarities or differences in biology, health, or disease.

Louis V. DePaolo, PhD, joined the RSB in 1994, after completing the NIH Grants Associates Program. He is the program director for a portfolio of grants in the areas of reproductive neuroendocrinology and pituitary function. Dr. DePaolo also serves as the research coordinator for both the SCCPRR and the NCPIR, and is director of the NICHD's CIR-LRP. 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 the 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 of the Department of Molecular Endocrinology at the Whittier Institute in La Jolla. His research background focuses on neuroendocrine control of female reproduction. Dr. DePaolo serves on the Student Affairs and Research Affairs Committees of the Endocrine Society, the NIH Staff Training in Extramural Programs

Committee, the NICHD Committee to Evaluate Centers and Networks, and the NICHD Committee to Redesign the Division of Scientific Review (DSR).

Estella C. Parrott, MD, MPH, joined the RSB in 1998, from the National Institute of Allergy and Infectious Diseases. She earned an undergraduate degree in biology from the City College of New York, her master's degree from the University of Chicago, and her degree in medicine from the University of Illinois Medical School, which was followed by a residency in obstetrics and gynecology. She is board-certified in obstetrics and gynecology and has a master's in public health from the George Washington University. Dr. Parrott serves as the program director for the Reproductive Medicine-Gynecology program and is responsible for administering a portfolio of research grants, research training, and career development projects that focus on the female factors of infertility, and on the gynecological aspects of reproductive tract diseases and disorders. She is the program officer for the RMN, RSDP, WRHR Career Development Centers, and administered the BIRCWH program until the spring of 2002. In addition, she is co-chair of the Career Development Subcommittee for the ORWH and a member of the NIH Coordinating Committee on Research on Women's Health. Prior to her appointment at the NIH, she held positions with the Food and Drug Administration and with the HRSA.

Tracy L. Rankin, PhD, joined the RSB in the fall of 2001, and serves as the director for the Male Fertility, Infertility, and Andrology Program. Her portfolio encompasses research into the mechanisms of spermatogenesis and sperm maturation, as well as into testicular and accessory gland functions in male reproduction. She is also serving as the committee coordinator for the RMN. Dr. Rankin received her undergraduate degree in biology from the University of Virginia, and her doctorate in cell biology from Vanderbilt University. She held postdoctoral positions at Tufts University, the Worcester Foundation for Biomedical Research, and at the National Institute of Diabetes and Digestive and Kidney Disorders before joining the RSB. Her research background includes epididymal sperm maturation, spermatogenesis, fertilization, and the structure and function of the mammalian zona pellucida.

Richard J. Tasca, PhD, 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 received his undergraduate degree in zoology at the University of Pennsylvania, and his graduate degree in mammalian developmental genetics from Temple University. He is the program director for the Preimplantation Development program and the Developmental Biology of Reproduction program and serves as the research coordinator for two National Cooperative Programs, one on Nonhuman *In Vitro* Fertilization and Preimplantation Development, and one on Mouse Sperm Cryopreservation. His research background is in the genetics, molecular biology, and nutrition of preimplantation embryos. He serves on the NIH Human ES Cell Implementation Group. He recently served as a member of the program committee, Society for the Study of Reproduction.

Susan Taymans, PhD, joined the RSB in November 1999. Dr. Taymans received her undergraduate degree from the University of Virginia, and her doctorate in molecular endocrinology from the University of Maryland. She held pre-doctoral and postdoctoral Intramural Research Training Award positions in the NICHD before coming to the extramural community. Her research background is in reproductive behavior, molecular endocrinology,

endocrine genetics, and positional cloning. She is the program director for the Reproductive Genetics portfolio, which encompasses research in sex determination, the genetics of reproductive aging, infertility genetics, including epidemiologic studies, and non-Mendelian inheritance in reproduction. She also handles the portfolio of ovarian basic science research grants. Dr. Taymans manages the RSB's institutional training grants (T32s) and serves on the NICHD's Training Policy Committee.

Donna L. Vogel, MD, PhD, left the RSB in October 2000, after 13 years of invaluable service. Until her departure, Dr. Vogel was the program director for the Reproductive Medicine (Andrology) portfolio. In addition, Dr. Vogel coordinated the special awards component of the Branch, served as the research coordinator for the RMN and the NCPIR, as program coordinator for the WRHR and BIRCWH programs, and the RSB Training Officer. She was co-chair of the Research Subcommittee, NIH Coordinating Committee on Research on Women's Health; a member of the Urology Subcommittee of the Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee; and on the Executive Committee, Reproductive Scientist Development Program.

Koji Yoshinaga, PhD, joined the RSB in 1978. He is the program director for a portfolio of grants in reproductive endocrinology. In addition, he was the research coordinator for the National Cooperative Program on Markers of Uterine Receptivity for Blastocyst Implantation and currently serves as the research coordinator of the Cooperative Program on Trophoblast-Maternal Tissue Interactions. He is the director of the Reproductive Sciences of the Americas Network project to enhance international collaboration in training and research in reproductive sciences among Pan American countries. He also serves as the research coordinator of the SCCPRR Research Focus Groups on Endometrium Function/Dysfunction and on Endometriosis. He received his bachelor's, master of science, and doctorate degrees from the University of Tokyo, and received postdoctoral training in the Training Program in Reproductive Physiology at the Worcester Foundation for Experimental Biology, as well as 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.

APPENDIX B: PUBLICATIONS BY RSB PERSONNEL: 1998-2002

(RSB staff names appear in bold.)

Leppert PC. (1998). The biochemistry and physiology of the uterine cervix during gestation and parturition. *Prenatal and Neonatal Medicine*, 3:103-106.

Leppert PC. (1998). Proliferation and apoptosis of fibroblasts and smooth muscle cells in rat uterine cervix throughout gestation and the effect of the antiprogestone, Onapristone. *American Journal of Obstetrics and Gynecology*, 178:713-25.

Leppert PC, Burtner C, and Raines D. (1998). Medicaid managed care partnerships to improve perinatal outcomes. *American Journal of Public Health Management and Practice*, 4:82-89.

Takamoto N, Yu SY, and **Leppert PC.** (1998). Cell death and proliferation and its relation to collagen degradation of uterine involution in rat. *Connective Tissue Research*, 37:163-175.

Elhassan YM, Lee CP, **Tasca RJ,** and Westhusin ME. (1999). The effect of NaCl-associated osmolarity changes on bovine embryo development in a completely defined culture medium supplemented with amino acids at oviductal fluid levels. *Biology of Reproduction*, 60:248.

Guzick DS, Carson SC, Coutifaris C, Overstreet JW, Factor-Litvak P, Steinkampf MP, Hill JA, Mastroianni L, Buster JE, Nakajima ST, **Vogel DL,** and Canfield RE. (1999). Efficacy of superovulation and intrauterine insemination in the treatment of infertility: results of a randomized, controlled multicenter study. *New England Journal of Medicine*, 340:177-83.

Paria BC, Zhao X, Das SK, Dey SK, and **Yoshinaga K.** (1999). Zona occludens-1 (ZO-1) and E-cadherin are coordinately expressed in the mouse uterus with the initiation of implantation and decidualization. *Developmental Biology*, 208:488-501.

Taymans SE, Kirschner LS, and Stratakis CA. (1999). Radiation hybrid mapping of chromosomal region 2p15-p16: integration of maps of expressed and polymorphic sequences at the Carney Complex (CNC) & Doyme's Honeycomb Retinal Dystrophy (DHRD) loci. *Genomics*, 56:344-349.

Vogel DL. (1999). NIH Funding and Career Development Opportunities for Women in Reproductive Immunology. *Annual Meeting of the American Society of Reproductive Immunology*, (Abstract).

DePaolo LV, Hinton BT and Brawn RE. (2000). Male contraception: views to the 21st century, Bethesda, MD, USA, 9-10 September, 1999. *Trends in Endocrinology and Metabolism*, 11:66-69.

Leppert PC, Kokenyesi R, Klemenich CA, and Fisher J. (2000). Further evidence of a decorin-collagen interaction in the desruption of cervical collagen fibers during rat gestation. *American Journal of Obstetrics and Gynecology*, 182:805-812.

Newbold RR, DiAugustine RP, Risinger JI, Everitt JI, Walmer DK, **Parrott EC**, and Dixon D. (2000). Advances in uterine leiomyoma research: conference overview, summary, and future research recommendations. *Environmental Health Perspectives*, 108(S5):769-773.

Stratakis CA, Lafferty A, **Taymans SE**, Gafni RI, Meck JM, and Blancato J. (2000). Anisomastia associated with interstitial duplication of chromosome 16, mental retardation, obesity, dysmorphic facies and digital anomalies: molecular mapping of a new syndrome by fluorescent *in situ* hybridization and microsatellites to 16q13 (D16S419-D16S503). *Journal of Clinical Endocrinology and Metabolism*, 85:3396-3401.

Stratakis CA, Papageorgiou T, Premkumar A, Pack S, Kirschner LS, **Taymans SE**, Zhuang Z, Oelkers WH, and Carney JA. (2000). Ovarian lesions in Carney complex: clinical genetics and possible predisposition to malignancy. *Journal of Clinical Endocrinology and Metabolism*, 85:4359-4366.

Yoshinaga K. (2000). Cellular and molecular events surrounding blastocyst implantation: report on a NICHD meeting, Bethesda, MD, USA, November 15-16, 1999. *Trends in Endocrinology and Metabolism*, 11:116-118.

Elhassan YM, Shin T, **Tasca RJ**, and Kraemer DC. (2001). *In vitro* culture of cloned and *in vitro* fertilized bovine embryos in a chemically defined culture medium. *Theriogenology*, 55:266.

Elhassan YM, Wu G, Leanez AC, **Tasca RJ**, Watson AJ, and Westhusin ME. (2001). Amino acid concentrations in fluids from the bovine oviduct and uterus and in KSOM-based culture media. *Theriogenology*, 55:1907-1918.

Lafferty AR, Torpy DJ, Stowasser M, **Taymans SE**, Lin JP, Huggard P, Gordon RD, and Stratakis CA. (2001). A novel genetic locus for low renin hypertension: familial hyperaldosteronism type II maps to chromosome 7 (7p22). *Journal of Medical Genetics*, 37:831-835.

Parrott EC, Santoro N, and **Leppert PC**. (2001). The ovary: genesis, function and failure. (Guest Editors). *Journal of The Society for Gynecologic Investigation*, 8(S1):1-68.

Parrott EC. (2001). Introduction to reproductive and related endocrine considerations: Selective Estrogen Receptor Modulators (SERMS). *Annals of the New York Academy of Sciences*, 949:235-236.

Rankin TL, O'Brien M, Lee E, Wigglesworth K, Eppig J, and Dean J. (2001). Defective zonae pellucidae in *Zp2*-null mice disrupt folliculogenesis, fertility, and development. *Development*, 128:1119-1126.

Soules SR, Sherman S, **Parrott E**, Rebar R, Santoro N, Utian W, and Woods N. (2001). Executive summary: stages of reproductive aging workshop. *Fertility and Sterility*, 76:874-878.

Stratakis CA, **Taymans SE**, Schteingart D, and Haddad BR. (2001). Segmental uniparental disomy (UPD) for 2p16 without clinical symptoms: implications for UPD and other genetic studies of chromosome 2. *Journal of Medical Genetics*, 38:106-109.

Tasca RJ. (2001). "The cell biology of preimplantation development." in *ART and the Human Blastocyst*. (Eds. David Gardner and Michelle Lane). Serono Symposia, USA, Norwell, MA, pp. 51-60.

Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, and **Yoshinaga K**. (2002). Embryo implantation. *Developmental Biology*, 15: 223:217-37.

Kirschner LS, Carney JA, Pack S, **Taymans SE**, Giatzakis C, Cho YS, Cho-Chung YS, and Stratakis CA. (2002). Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit (*PRKA1A*) in patients with the Carney Complex. *Nature Genetics*, 26:89-92.

Kuo SM, Stout A, Wactawski-Wende J, and **Leppert PC**. (2002). Ascorbic acid status in postmenopausal women with hormone replacement therapy. *Maturitas*, 41:45-50.

Yoshinaga K and **Parrott EC**. (2002). Endometriosis: emerging research and intervention strategies. (Co-editors). *Annals of the New York Academy of Sciences*, 955:1-406.

DePaolo LV and **Leppert PC**. (In Press). Providing a research and research training infrastructure for clinical research in the reproductive sciences. *American Journal of Obstetrics and Gynecology*.

Jensen JT, Schwino KM, Zelinski-Wooten MB, Conti M, **DePaolo LV**, and Stouffer RL. (In Press). Phosphodiesterase 3 inhibitors selectively block the spontaneous resumption of meiosis by macaque oocytes *in vitro*. *Human Reproduction*.

Leppert P and Artal R. (In Press). A Survey of Past Obstetrics and Gynecology Research Fellows. *Journal of the Society for Gynecologic Investigation*.

APPENDIX C: RSB STAFF ACTIVITIES

INVITED LECTURES

Phyllis C. Leppert

- *Science for the Millennium*, Upstate Perinatal Research Meeting, Ithaca, New York, January 2000.
- *Hormone Replacement in Women*, University of Puerto Rico, San Juan, January 2000.
- President's Panel: *Ethics in Research*, American College of Obstetricians and Gynecologists (ACOG) Annual Meeting, May 2000.
- *How to Conduct a Research Project*, and *Cutting Edge Research at NIH*, ACOG Annual Meeting Workshops, May 2000.
- *NICHD People and Programs*, Society of the Study of Reproduction, Ottawa, July 2001.
- *Research Opportunities in Reproductive Sciences*, American Association of Obstetrics and Gynecology Foundation Fellows Retreat, Kohler, Wisconsin, August 2001.
- *Basic Science of Uterine Leiomyomas*, Reproductive Endocrinology and Infertility Unit, Pediatric and Reproductive Endocrinology Branch, September 2001.
- *Recent Research Advances in Reproductive Sciences*, Grand Rounds-Department of Medicine, Virginia Commonwealth University, Richmond, Virginia, October 2001.
- *New Theories of Etiology of Fibroids*, University of Puerto Rico, San Juan, January 2002.
- RSB Directors' Meeting: Oregon Regional Primate Facility, Beaverton, Oregon, May 2000; Bethesda, Maryland, May, 2001; and University of California, San Diego, May 2002.

Estella C. Parrott

- *Fibroids: Is Surgery the Only Solution?* Health Issues and Concerns of Women of Color: A Call to Action, Washington, DC, April 1999.
- *Reproductive Medicine Research Update*, National Medical Association Meeting, Las Vegas, Nevada, August 1999.
- *NICHD Funding and Training Opportunities*, 4th International Conference on Postoperative Healing and Adhesions, Fort Lauderdale, Florida, October 1999.
- *NICHD Funding and Training Opportunities*, Berlex Foundation Faculty Development Course, Captiva Island, Florida, February 2000.
- *Cutting-edge Research in Obstetrics and Gynecology at the NIH*, NIH Symposium at the ACOG Annual Clinical Meeting, Chicago, Illinois, April 2001.
- *Interdisciplinary Research: Models and Policy Approaches*, Society for Women's Health Research Scientific Advisory Committee Meeting, Washington, DC, July 2001.

Richard J. Tasca

The Cell Biology of Preimplantation Development, at Sero Symposia, USA, ART and the Human Blastocyst, Dana Point, California, March 2000.

Koji Yoshinaga

Exploring Opportunities of Collaboration in Research and Development in Reproductive Health Between Research Institutions in Latin America and Industry, Reproductive Sciences of the Americas Network Symposium, Curitiba, Brazil, April 2001.

LIAISON ACTIVITIES**Phyllis Leppert**

- ACOG Gynecologic Practice Committee, 2000
- ACOG Genetics Committee, 2001-2002
- DHHS Assisted Reproductive Technology (ART) Working Group
- NICHD Contraceptive Microbicide Subcommittee
- NICHD Data and Safety Monitoring Board for Clinical Center Protocol; Raloxiphen in the Treatment of Endometriosis Pain
- NICHD Fertility and Early Pregnancy Working Group, National Children's Study
- Quarterly News Briefs, American Society of Reproductive Medicine Newsletter, 2001-2002
- Society for Gynecologic Investigation, Program Committee, 2000-2002
- Trans-NIH Endocrinology, Metabolism, and Reproductive Sciences Integrated Review Group, Steering Committee
- NICHD CIR-LRP Selection Committee

Lou De Paolo

- American Neuroendocrine Society, Program Committee
- The Endocrine Society, Student Affairs and Research Affairs Committees
- NICHD Committee to Evaluate Centers and Networks
- NICHD Committee to Redesign the DSR
- NIH Endocrinology, Metabolism, and Reproductive Sciences Integrated Review Group, Study Section Boundaries Team
- NIH Staff Training in Extramural Programs Committee
- Society for the Study of Reproduction, Education Committee
- Trans-NIH Endocrine Group

Estella Parrott

- ACOG, Gynecologic Practice Committee
- NICHD Advisory Group on Health Disparities
- NICHD CIR-LRP, Reviewer
- NICHD Research Supplements for Underrepresented Minorities, Reviewer
- NIH Coordinating Committee on Research on Women's Health

- NIH EA Advisory Board
- ORWH Ad Hoc Working Group Review Committee for the Research Enhancement Awards Program
- ORWH Task Force, Recruitment and Retention in Clinical Trials Workshop

Tracy Rankin

NICHD representative on the Urology Subcommittee of the Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee

Richard Tasca

- NIH Human ES Cell Implementation Group; Society for the Study of Reproduction, Program Committee, 1999-2001
- 1998 NIH Organizing Committee for *Priority Setting for Mouse Genomics and Genetics Resources*

Susan Taymans

NICHD Training Policy Committee

Koji Yoshinaga

Society for the Study of Reproduction, Program Committee, 2000-2002

RSB-SUPPORTED CONFERENCES AND WORKSHOPS

- *Becoming a Mother in the Late Reproductive Years*, Bethesda, MD, May 4-5, 1998
- FIR, Woods Hole, MA, May 26-July 5, 1998
- FIR Symposium, *The Legacy of Advanced Training in Fertilization and Gamete Physiology*, Woods Hole, MA, June 24-27, 1998
- XV Testis Workshop, Louisville, KY, April 7-10, 1999
- FIR, Woods Hole, MA, May 24-July 4, 1999
- FIR Symposium, *Decisions in Reproduction: Critical Functional Events in Pregnancy*, Woods Hole, MA, June 30-July 2, 1999
- *Male Contraception: Views to the 21st Century*, Bethesda, MD, September 9-10, 1999
- *Women's Health and the Environment: The Next Century-Advances in Uterine Leiomyoma Research*, Research Triangle Park, NC, October 7-8, 1999
- Conference on Cellular and Molecular Events Surrounding Blastocyst Implantation, Bethesda, MD, November 15-16, 1999
- *The Ovary: Genesis, Function, and Failure*, Bethesda, MD, March 30-31, 2000
- Workshop on the Outcome of Offspring Born as a Result of ICSI, Bethesda, MD, April 11, 2000
- NIH Workshop on SERMs, Bethesda, MD, April 26-28, 2000
- FIR Course, Woods Hole, MA, May 21-June 30, 2000
- FIR Symposium, *The Oocyte and Human Reproduction*, Boston, MA, June 15-17, 2000

- *Polycystic Ovary Syndrome: Basic Biology and Clinical Intervention*, Research Triangle Park, NC, September 17-20, 2000
- Information/Technical Assistance Preapplication Workshop for Reproductive Science Research Centers at Minority Institutions Program, Bethesda, MD, February 5, 2001
- *Effects of Maternal Health on Reproductive Events Leading to Adult Diseases and Disorders in Offspring*, Bethesda, MD, February 28, 2001
- NIH Workshop on Endometriosis: Emerging Research and Intervention Strategies, Bethesda, MD, April 9-10, 2001
- 6th International Conference on the Extracellular Matrix of the Female Reproductive Tract, Bethesda, MD, May 12-14, 2001
- Research Meeting of the SCCPRR, Bethesda, MD, May 14-16, 2001
- RSB Center Director's Meeting, Bethesda, MD, May 17, 2001
- WRHR Center Directors' Meeting, Bethesda, MD, May 18, 2001
- FIR Course, Woods Hole, MA, May 20-July 1, 2001
- FIR Symposium, *Reproductive Genetics, Genomics, and Proteomics*, Cambridge, MA, June 29-July 1, 2001
- Stages of Reproductive Aging (STRAW) Workshop, Park City, UT, July 23-24, 2001
- *Chronic Pelvic Pain: Pathogenic Mechanisms, Treatment Innovations, and Research Innovations*, Bethesda, MD, April 8-9, 2002
- *Apoptosis in the Male Reproductive Tract*, Bethesda, MD, March 21-22, 2002
- *Imprinting in Gametogenesis and Development*, Bethesda, MD, April 30, 2002
- RSB Center Director's Meeting, San Diego, CA, May 9, 2002
- Preapplication Workshop for Reproductive Sciences Research Centers, Bethesda, MD, May 20, 2002

APPENDIX D: RSB-SPONSORED RFAs AND PAs

RFA: HD-98-012	Mouse Sperm Cryopreservation
RFA: HD-98-013	SCCPRR
PA: PA-98-112	Vulvodynia: Systematic Epidemiologic, Etiologic, or Therapeutic Studies
RFA: HD-99-001	WRHR Career Development Centers
RFA: HD-99-003	Basic Science Research on Female Pelvic Floor Disorders
RFA: HD-99-005	Cooperative Multi-center RMN
RFA: OD-99-008	BIRCWH
RFA: HD-99-009	SCCPRR
RFA: HD-00-008	Pathophysiology, Epidemiology, and Treatment of Vulvodynia
RFA: HD-00-019	Cooperative Reproductive Science Research Centers at Minority Institutions
RFA: HD-00-022	SCCPRR
RFA: HD-01-001	Cooperative Specialized Infertility Research Centers
PA: PA-01-005	Reproductive Genetics
RFA: HD-01-012	Cooperative Program on Trophoblast-Maternal Tissue Interactions
RFA: HD-01-020	Mouse Phenotyping: Developmental and Fertility Defects (with the Developmental Biology, Genetics, and Teratology Branch, NICHD)
RFA: HD01-023	SCCPRR
PA: PA-01-067	Biology of the Menopausal Process and Associated Health Conditions During and After Menopause
RFA: OD-02-001	BIRCWH
RFA: OD-02-002	Specialized Centers of Research on Sex and Gender Factors Affecting Women's Health
RFA: HD-02-012	Cooperative Reproductive Science Research Centers at Minority Institutions
RFA: HD-02-018	Female Health and Egg Quality
PA: PA-02-090	Vulvodynia: Systematic Epidemiologic, Etiologic, or Therapeutic Studies