Reproductive Sciences Branch
NICHD

Report to the NACHHD Council
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EXECUTIVE SUMMARY

The Reproductive Sciences Branch (RSB), within the Center for Population Research of the National Institute of Child Health and Human Development (NICHD), is pleased to present its report to the National Advisory Child Health and Human Development (NACHHD) Council. This report, which covers activities from 2002 through 2006, highlights some of the most exciting research advances made by RSB-funded scientists, summarizes the Branch's special research programs and research training and career development activities, explains the expert panel discussions and portfolio review, and describes the Branch's priorities for the next four years.

The Branch's portfolio is currently distributed among eight areas:

- Reproductive Neuroendocrinology
- Reproductive Endocrinology and Immunology
- Reproductive Genetics and Epigenetics
- Preimplantation Genetics and Development
- Ovarian Biology
- Reproductive Medicine Gynecology
- Male Reproductive Health
- Interdisciplinary Research in Reproduction

Highlighted scientific advances from the Branch include:

- Innovations in understanding how the brain regulates puberty and ovulation
- Identification of key molecules involved in ovarian follicle development, early development of a fertilized egg, and the maintenance and differentiation of spermatogonial stem cells
- Demonstration of unique patterns of gene expression in individual human embryonic stem cell lines approved for use by the Federal Government
- Observation that undifferentiated mouse embryonic stem cells can develop into germ cells
- Definition of new properties of gametes, including their ability to control nuclear remodeling and their role in sex determination
- New insights into the relationship between obesity, androgen excess, and the polycystic ovary syndrome (PCOS) in adolescents and the implications for predicting the prevalence of metabolic syndrome in adulthood
- Finding that the anti-estrogen clomiphene is superior to metformin, an insulin-sensitizing drug, for the treatment of infertility (e.g., ovulation induction, conception, live birth rate) in women with PCOS
- Identification of potential molecular and genetic markers and treatments for uterine fibroids
- Use of DNA microarray technology to assess global gene-expression patterns during implantation, in endometriosis, and for women with pelvic organ prolapse

As part of the NICHD's continued efforts to increase accountability and transparency in strategic planning, the RSB sought advice and feedback on future directions for the Branch from an expert panel, which consisted of top scientists in reproductive biology and medicine, representatives from science advocacy groups, and two liaisons to the NACHHD Council (see Appendix F).

The expert panel participated in an extensive review and analysis of the Branch's current research portfolio and activities, and training and funding trends. After discussing three overarching questions, the panel recommended:

- Revising the Branch mission statement
- Taking a greater initiative in publicizing the Branch mission
- Focusing more on public health issues beyond infertility
- Placing greater emphasis on the etiology of complex diseases and disorders, particularly those with genetic, epigenetic, and environmental components
- Maintaining the balance between investigator-initiated research and center programs
- Increasing collaboration with other NICHD Branches and other NIH Institutes, Centers, and Offices

In an effort to strengthen the Branch's focus on issues of public health significance, the RSB established two programs that cross-cut the various research portfolios: Fertility Preservation and Preconception Care. In addition, the Branch identified key priority areas related to research, technology/bioinformatics, and investigator recruitment and retention for the next four years, including:

- Expansion of technologic resources for scientists in the field
- Increased efforts encouraging young investigators to develop an interest in careers in the reproductive sciences
- Establishment/expansion of programs to retain physician-scientists in research careers
- Continued expansion of projects related to and with an emphasis on research addressing the etiology of women's reproductive diseases
- Greater attention to diagnosing reproductive and gynecologic diseases of the pre- and post-pubertal adolescent
- Promotion of the concept of male reproductive health
- Support for studies that move beyond the correlation of a causative gene with a reproductive disease or disorder
- Encouragement of research on all aspects of stem cell biology
- Pursuit of investigations at the intersection of the immune and endocrine systems

In addition to the portfolio review conducted by the expert panel, the Branch also went through an in-depth analysis of its portfolio in terms of the success rate of grant applications submitted in specific science areas for fiscal year 2001 through fiscal year 2006. Branch staff also sought to determine how the reorganization of study sections by the Center for Scientific Review (CSR) impacted the success rate of grant applications in the reproductive sciences. The RSB also evaluated the impact of the NIH budget on its support of new investigators to determine how to increase such support within the constraints of the current fiscal climate.

INTRODUCTION TO THE BRANCH

The cycle of life for any given species hinges on reproduction, as each generation develops and matures to produce the succeeding generation. The process of human reproduction, indeed propagation of the human species, depends on a cascade of precisely timed, integrated events that involve such diverse tissues as gametes (e.g., sperm and eggs), neurons, steroid-producing cells in the gonads, and the uterine endometrium. These events do not operate in isolation, but rather are modulated by what a person eats, what a person does, and how the person does it. The challenge is to understand the complex process of reproduction in the context of 21st century lifestyles to enable each individual to control his or her own fertility and maintain optimum reproductive health.

With this in mind, the RSB encourages and supports 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. The RSB supports a comprehensive program of basic, applied, clinical, and translational studies to increase understanding of normal reproduction and reproductive pathophysiology, and to develop more effective strategies to diagnose, treat, and prevent conditions that compromise reproductive health. The RSB also supports training and career development opportunities in the reproductive sciences for individuals from the undergraduate through post-graduate levels. The Branch is the single largest funding source for reproduction research in the Federal Government, providing oversight of more than 500 grant awards that total nearly $150 million (see Figure 5, Figure 6, Figure 7, Figure 8, and Figure 9). The Branch mission, priorities, and activities are disseminated through its Web site: http://www.nichd.nih.gov/about/org/cpr/rs/.

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The next section of this report presents research highlights organized into the broad areas of: central control of reproductive processes (Reproductive Neuroendocrinology); gender specificity (Female Reproduction, Male Reproduction); and early developmental aspects of reproduction (Developmental Biology of Reproduction).

HIGHLIGHTS OF SCIENTIFIC ADVANCES

REPRODUCTIVE NEUROENDOCRINOLOGY

The pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), regulate the production of steroid hormones and mature gametes. Both are released episodically in response to gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. Deviations in the timing of GnRH pulses, and consequent changes in timing of LH and FSH pulses, can result in infertility. Therefore, it is essential to understand the biochemical and molecular processes that regulate pulsatile secretion of GnRH and responsiveness of the pituitary gonadotropes to GnRH.

Role of Fibroblast Growth Factors (FGF) in Development of the GnRH Neuronal System

Disruption of the birth and migration or axonal targeting of GnRH neurons can significantly impair reproduction. A subpopulation of GnRH neurons expresses FGF receptors, which have profound effects on the developing nervous system. A study by RSB-funded investigators found that, in a GnRH cell line, mutant FGF-1 receptor diminished both responsiveness to FGF and neurite outgrowth. Despite normal distribution of GnRH neurons, fewer GnRH cells expressed the mutant FGF-1 receptor specifically in GnRH neurons in transgenic mice. These mice were initially fertile, but later had delayed puberty, smaller litters, and early reproductive senescence, demonstrating that FGF signaling is important for the survival of GnRH neurons. Interestingly, mutations in the FGF-1 receptor are found in patients with Kallman's syndrome, a human disorder associated with irreversible puberty delay, infertility, and low serum gonadotropin levels. Thus, knowledge gained from mouse models could present new treatment opportunities for those with Kallman's syndrome.

New Therapeutic Strategy for Treatment of Hypothalamic Hypogonadism (HH)

Individuals with HH fail to undergo puberty due to inappropriate GnRH secretion. Some forms of congenital HH result from GnRH-receptor (GnRHR) mutations that reduce receptor expression at the cell surface. When cells with such mutant GnRHRs were treated with IN3, a non-peptide GnRH antagonist that penetrates the cell, the biological activity of GnRH was restored. IN3 corrected the GnRHR mutant structure to increase its expression at the cell surface. Analogs of GnRH that did not penetrate the membrane were ineffective. Agents that "rescue" mutant GnRHR might offer a novel way to treat HH caused by GnRHR mutations.

Dual-Phenotype Gamma-Aminobutyric Acid (GABA)-Glutamate Neurons Regulate Reproductive Function

The neurotransmitters GABA and glutamate inhibit and stimulate, respectively, GnRH release from neurons in the preoptic area. Contrary to long-held assumptions, findings from research conducted by RSB-funded scientists showed that virtually all neurons in the preoptic area of female rats can synthesize and release both GABA and glutamate. Moreover, they found that these neurons make direct contact with GnRH neurons and are estrogen responsive. At proestrus, GABA neuronal activity decreased while glutamate neuronal activity increased in these dual-phenotype neurons. The brains of female rats had 2.5 times as many dual-phenotype neurons as male rat brains, accounting for the inability of males to mount an LH surge, even when given estrogen. These findings provide compelling evidence that GABA and glutamate are released from the same neurons located in the preoptic region. In addition, these neurons are targets for estrogen and exhibit a sexual dimorphism. This important population of neurons mediates estrogen stimulation of the female-specific LH surge that triggers ovulation.

Ghrelin Suppresses LH Release in Primates

The peptide ghrelin, which is secreted primarily by the stomach, strongly stimulates food intake. Prolonged food restriction increases the level of circulating ghrelin and suppresses reproduction. Interestingly, ghrelin levels rise in humans who have anorexia nervosa, a condition in which severe nutrient deprivation is often accompanied by amenorrhea and suppressed LH pulsatility. New RSB-funded research shows that infusion of human ghrelin markedly decreased LH pulse frequency in adult rhesus monkeys lacking ovaries. Likewise, ghrelin might suppress LH pulsatility in women with anorexia nervosa, leading to the amenorrhea common among women with this condition.

FEMALE REPRODUCTION

The female reproductive system is dynamic, involving cyclic fluctuations in the secretion of hormones from the brain, anterior pituitary gland, and the ovaries. These hormones have a number of functions in the body, including (but not limited to):

- Promoting the development of ovarian follicles
- Promoting oocyte growth, differentiation, and maturation into a fertilizable egg
- Releasing an egg through the process of ovulation
- Preparing the reproductive tract for implantation of the embryo

To understand the physiology and pathophysiology of human female reproduction, it is critical to delineate the mechanisms that govern this finely tuned sequence of hormonal changes and their effects using various molecular, cellular, and animal models.

Mechanism of Gonadotropin Action

RSB-funded scientists have produced high-resolution structures of human chorionic gonadotropin (hCG)—a molecule that interacts with LH receptors—and FSH; investigators also identified the parts of these hormones that enable them to distinguish between receptors for LH and FSH. The hormones LH, hCG, and FSH have an identical alpha-subunit, which is stabilized

by the surrounding beta-subunit that is primarily responsible for receptor-binding specificity. The researchers created a new model of the extracellular and transmembrane domains that offers a simple explanation for how these hormones stimulate gonadal function. The model has already enabled the scientists to design new antagonist hormone analogs and may help to explain the constitutive activities of several receptor mutations that cause precocious puberty. These types of studies can drive the design of gonadotropin analogs and inhibitors for use in basic science and, potentially, in contraception or in the treatment of infertility.

The Interplay between Oocytes and Follicles

The mammalian oocyte and its companion granulosa cells are interdependent partners in follicle development, cooperating to release an oocyte that is ready for fertilization and embryogenesis. In fact, the closely related oocyte factors GDF-9 and BMP-15 critically regulate their surrounding follicle. Data from mice and sheep indicate that, in the absence of these factors, follicles fail to develop, causing infertility. Paradoxically, when these factors are present, but at lower-than-normal levels, more immature follicles ovulate, increasing fertility. An RSB-supported investigator found that, when expressed together, GDF-9 and BMP-15 form heterodimers that impair the process critical for making mature forms of both proteins. Thus, it is the secretion, not the activity of these factors that is critical to fertility in mutant sheep and mice.

Another RSB-funded scientist recently found mutant GDF-9 in a family prone to twinning. This finding further demonstrates the importance of this pathway in human fertility. Because GDF-9 and BMP15 are oocyte specific, and systemic knockout mice showed no effects other than infertility, these molecules present exciting targets for fertility regulation.

Regulation of Oocyte Meiosis

In vertebrates, oocyte development begins in the embryo, but then arrests at birth; the oocytes are held suspended in the first stage of meiosis. As oocytes mature, their intrinsic restraint on meiosis must be bolstered by inhibitory signals from surrounding somatic cells. Oocyte receptors sense the signal from the somatic cells, suspending the oocyte in mid-meiosis until the LH surge at ovulation overcomes the meiotic block. RSB-funded scientists recently identified the oocyte receptor as Gpr3, a G-protein coupled receptor that is highly expressed in oocytes and that activates G_s, which prevents meiosis. Mature oocytes from mice that lack Gpr3 spontaneously resumed meiosis; when Gpr3 was replaced, the oocytes again stopped in mid-meiosis until they were exposed to an ovulatory surge of hormones, at which point meiosis resumed. With the knowledge that Gpr3 is the oocyte receptor that maintains meiotic arrest in mature oocytes, researchers now have a firm base for discovering the corresponding inhibitory signal from the granulosa cells, as well as how the signal is regulated by hormones throughout the menstrual cycle. Understanding the regulation of meiosis in oocytes may help explain female fertility, female reproductive aging, and pregnancy loss because meiotic errors, such as aneuploidy, are the primary cause of miscarriages, especially as women age.

Predictors of Egg Quality Found in Surrounding Cells

Reliable identification of “high quality” eggs would be of great benefit to assisted reproduction technologies (ART) because it would limit the number of embryos needed for transfer to the uterus, while maintaining a satisfactory pregnancy rate. This knowledge would decrease the

incidence of multiple births, which are risky for the infants as well as for the mothers. Currently, ART involves embryos selected by appearance and by the amount of fragmentation; but about 40 percent of human embryos that look normal have chromosomal abnormalities.

Two recent papers by RSB-funded scientists show that certain molecular markers in the cumulus (somatic) cells that surround human eggs are good non-invasive predictors of successful fertilization and healthy embryos in *in vitro* fertilization (IVF) clinics. These markers include Gremlin and Pentraxin 3, both of which are under the influence of GDF-9, an important oocyte factor that regulates follicle growth. Use of these markers for oocyte and embryo selection could allow fewer, but healthier embryos for transfer to initiate pregnancy during IVF procedures.

Gene Profiling during the Window of Human Implantation

A scholar in the Women's Reproductive Health Research (WRHR) Career Development Program working in the Stanford Specialized Cooperative Center provided a breakthrough upon completing large-scale, microarray 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 that were 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 that could lead to improved ART.

Leiomyomata Uteri (Uterine Fibroids)

Leiomyomata, or uterine fibroids, are the most common benign tumors in reproductive-aged women. The leading indication for hysterectomy in the United States, uterine fibroids are two to three times more likely to occur in African American women than in Caucasian women. The mechanisms that mediate the growth and pathogenesis of uterine fibroids are not completely understood. Treatment with a GnRH analog (GnRHa) induces regression of uterine fibroids. Researchers have used gene-expression studies of leiomyoma and myometrial smooth muscle cells from GnRHa-treated women to identify members of several gene families that are important for transcription, translation, signal transduction, extracellular matrix turnover, and apoptosis. These genes may provide targets for novel interventional strategies in the treatment of fibroids.

Effective alternatives to hysterectomy for uterine fibroids would also be very valuable. To this end, several RSB-funded investigators are evaluating conservative therapeutic agents, such as mifepristone, which block progesterone receptors, as an alternative to surgery. Studies show that mifepristone treatment in pre-menopausal women who have large, symptomatic leiomyoma significantly decreased mean uterine volume and leiomyoma-related symptoms. Further studies will assess the long-term safety and efficacy of mifepristone for treating fibroids.

Researchers are aware that the growth and progression of uterine fibroids is dependent on estrogen. Polymorphisms in the gene that encodes the enzyme catechol-O-methyltransferase (COMT), which is essential to estrogen metabolism, can therefore affect a woman's risk for developing fibroids. One RSB-funded scientist found that a COMT polymorphism associated with high estrogen bioactivity and, therefore, an increased risk of fibroids is significantly more

prevalent in African American women than in Caucasian women. This finding could explain the racial disparity in the incidence of uterine fibroids. It may also provide a new target for specific interventions.

Pelvic Floor Dysfunction

During their lifetimes, women have a risk of up to 11 percent that they will require surgery for a pelvic floor disorder, such as pelvic organ prolapse or incontinence. Vaginal childbirth is a major risk factor for pelvic floor dysfunction. Animal models suggest that damage results from excessive nerve compression and stretch, and that the resulting nerve-injury might weaken the muscles, ligaments, and fascia of the pelvic floor. RSB-funded investigators found that, contrary to common belief, the human levator ani (LA) muscle, a major muscle of the pelvic floor along with the coccygeus, is not innervated by the pudendal nerve, but by a nerve originating from the sacral nerve roots. Previous studies had implicated the pudendal nerve in prolapse.

These investigators also established a squirrel monkey model for studying childbirth-related neuropathy of the LA muscles as a cause of pelvic organ prolapse. Using microarray analysis of gene expression in the pubococcygeus muscle of patients with advanced pelvic organ prolapse and controls, researchers identified many candidate genes, particularly those that encode structural proteins and extracellular matrix proteins. In the future, these techniques may allow health care providers to identify women at increased risk for urinary incontinence or pelvic organ prolapse based on gene expression.

The risk of pelvic organ prolapse increases with age and jumps dramatically at menopause, perhaps due to the decrease in ovarian steroids. Results from a study of rhesus macaques suggest that the proteinase inhibitor, cystatin C, protects the structural integrity of the vaginal wall. Findings also suggest that decreased estrogen secretion at menopause might weaken the pelvic floor by reducing the levels of cystatin C.

Urinary incontinence, one type of pelvic floor disorder, can be treated surgically using a fascia "sling" procedure, but this treatment sometimes causes patients to have trouble emptying their bladders. In an RSB-funded quality-of-life survey of women who underwent a sling procedure for urinary incontinence, 80 percent of the patients reported some improvement in urine leakage 12 months after surgery, and slightly more than 90 percent were satisfied with their overall progress four years after the surgery. Although some patients reported trouble emptying their bladders after the surgery, the percentage was actually lower than that reported before the surgery.

Polycystic Ovary Syndrome (PCOS)

Almost seven percent of reproductive-aged women suffer from androgen excess, presenting as hirsutism (excess body hair), thinning scalp hair, acne, and ovulatory dysfunction. RSB-funded scientists reported that, in comparison to the general population, women with androgen excess were twice as likely to be obese. In 80 percent of these women, the androgen excess was linked to PCOS; intervention studies indicate that suppressive hormone therapy reduced hirsutism, menstrual dysfunction, and acne in more than 80 percent of these patients. These data show that PCOS is the most common cause of androgen excess.

In a retrospective study, RSB-funded investigators found that the prevalence of the metabolic syndrome in women with PCOS (43 percent) is nearly two-fold higher than the age-adjusted prevalence rate of women nationally. The metabolic syndrome includes risk for developing type 2 diabetes mellitus and cardiovascular disease. The results suggest that clinicians may want to consider screening for metabolic syndrome in all women with PCOS, given its prevalence and its implications for serious cardiac disease.

Familial aggregation of PCOS also suggests a genetic contribution to the condition. Investigators supported by the NICHD and the NIH Office of Research on Women's Health (ORWH) found that sisters of women who had PCOS were more likely to have lipid abnormalities and the metabolic syndrome than normal controls. This study demonstrates the importance of heredity in reviewing long-term risk factors for abnormal lipid metabolism in sisters of women with PCOS.

MALE REPRODUCTION

The male reproductive system is a dynamic, interconnected system of endocrine and support organs. The adult testes consist of germ cells, in all stages of development, and the somatic Leydig and Sertoli cells, which respond to gonadotropins (LH and FSH) from the pituitary gland. At puberty, increases in circulating FSH levels cause Sertoli cells to multiply greatly; subsequently, the Sertoli cells regulate sperm production (spermatogenesis). Leydig cells respond to LH by synthesizing and secreting testosterone. Testosterone is critical for optimal sperm formation and maturation, maintenance of the male sex accessory glands, and normal sexual function.

During the last four years, the Branch's male reproductive health portfolio has grown significantly and advances have been made in all areas supported by the program. The following section highlights the most significant advances, focusing on four areas: Spermatogonial stem cell biology, sperm motility, capacitation (e.g., acquisition by sperm of the ability to fertilize eggs), and global analysis of testicular gene expression.

Spermatogonial Stem Cells

Sperm develop from spermatogonial stem cells in the testes. These stem cells, which continuously renew themselves by mitosis, can enter a pathway that differentiates them to form mature sperm. The identification of the molecular switch between maintenance of sperm in the undifferentiated state or those committed to the differentiation pathway remains one of the unsolved puzzles of stem cell biology. Studies in the model organism *Drosophila* (fruit flies) provide some insight into this process.

In male *Drosophila*, the germ-line stem cells attach to a group of somatic support cells called "the hub" or the stem cell "niche." A recently discovered ligand, Unpaired, is uniquely expressed in the hub. Results from recent studies also suggest that Unpaired acts through the JAK-STAT signaling pathway to promote self-renewal of the germ-line stem cells. In testes depleted of STAT, the stem cells all differentiate into spermatogonia; but, when STAT signaling is restored, these same spermatogonia "dedifferentiate" back into testes stem cells to repopulate

the stem cell niche. Based on these findings, then, differentiation is *not* irreversible, as had been assumed, at least in *Drosophila*. Continued studies of this model will allow researchers to identify key proteins that drive differentiated cells back to an undifferentiated state. Such knowledge could also apply to mammals because JAK-STAT signaling is required for the maintenance of mammalian embryonic stem cells.

RSB-funded scientists identified the gene *Plzf* as a key player in maintenance of mammalian spermatogonial stem cells. Male mice with a targeted knockout of *Plfz* were sterile and had a phenotype similar to that seen in mice with a spontaneous mutation, termed *luxoid*. This work also isolated a frame-shift mutation in *Plzf* in *luxoid* mutants. *Plfz* and *luxoid* male mice are sterile due to a depletion of stem cells from the testes. Thus, *Plzf* is one factor that maintains undifferentiated stem cells in the testes, providing a cellular reservoir for spermatogenesis throughout adult life. This finding may be the first mammalian protein reported to play a role in spermatogonial stem cell maintenance.

In a recent breakthrough, RSB-funded scientists developed a culture medium containing the precise combination of cellular growth factors needed for mouse spermatogonial stem cells to reproduce themselves *in vitro*. This finding will allow for the successful culture and *in vitro* manipulation of these cells. The cells remained undifferentiated in culture for several months, but successfully restarted spermatogenesis when transplanted into sterile mice. This advance opens the door to more targeted experimental manipulations of these cells and provides further insight into the unique biology of stem cells.

Sperm Motility and Capacitation

Adenylyl cyclases produce cyclic adenosine monophosphate (cAMP), a molecule that transmits instructions received from the cell surface to the cell nucleus, ultimately influencing target gene expression. Classical cyclases span the cell membrane, but a unique "soluble" adenylyl cyclase (sAC) is abundant in spermatozoa. Unlike membrane cyclases, sAC is activated directly by bicarbonate and Ca^{+2} ions, which are critical to inducing sperm motility, capacitation, and the acrosome reaction (process that releases enzymes from the sperm head). RSB-funded scientists discovered that male mice lacking the sAC gene are completely sterile because the spermatozoa can not move, but noted no other abnormalities in the testes or epididymis. When the investigators supplied cAMP, the sperm became motile. Further investigations showed that sAC is necessary for the molecular changes that occur in tyrosine phosphorylation, which takes place during capacitation, but is not necessary for either hyperactivated motility or the acrosome reaction; however, both processes are necessary for sperm to penetrate the egg. These latter observations were made possible through the development of a specific, small-molecule inhibitor of sAC, called KH7. Treating normal sperm with KH7 inhibited *in vitro* fertilization, but did so without poisoning the sperm because they recovered their fertilizing ability after co-incubation with cAMP.

cAMP also modulates sperm motility by stimulating the enzyme protein kinase A (PKA). PKA consists of regulatory subunits (RI or RII subunits) and catalytic subunits (C subunits). Surprisingly, male mice lacking the RII subunit are fertile, meaning that RII-dependent localization of the C subunit is not necessary for sperm motility. However, mice that lacked functional C subunits specific to mature sperm ($C_{\alpha 2}$) were infertile because their sperm failed to

hyperactivate and become fully capacitated. Calcium (Ca^{+2}) entry into $C_{\alpha 2}$ mutant sperm was also compromised, so the sperm were not fully activated for fertilization.

Even though hyperactivation of sperm motility is not dependent on sAC or on the cAMP it generates, the process does require sperm-specific calcium channels, the Catspers. CatSper1 is essential for calcium entry into sperm and resultant hyperactivated movement; however, the absence of CatSper1 does not affect capacitation or sperm motility. The absence of CatSper2, a related calcium channel, also causes male sterility because the sperm fail to hyperactivate. It is unclear whether CatSper1 and CatSper2 are subunits of the same calcium channel or are two separate channels; regardless, though, they are absolutely required for sperm to acquire full fertilizing capability. The CatSpers seem to be downstream of sAC and PKA in the molecular cascade that leads to full sperm capacitation and hyperactivated motility.

Testicular Gene Expression: Datasets and Resources

Several RSB-funded groups adapted microarray technology to define the entire profile of genes expressed at specific points in testes development and in spermatogenesis. One of the more surprising findings was the large number of genes involved in testes development, particularly the development that occurs after the onset of meiosis at the start of puberty. One study estimated that approximately 4 percent of the murine genome is dedicated to male germ cell-specific genes, most involved in meiosis or post-meiotic processes. An estimated 58 percent of the mouse genome is involved in testes development from birth to adulthood. Several other studies used microarray analysis to identify genes responsive to either FSH or testosterone in the testes. The predominant trend was up-regulation of genes in response to FSH and down-regulation of genes in response to testosterone.

All of these datasets are now available to investigators through the National Center for Biotechnology Information Gene Expression Omnibus, or through the Mammalian Reproductive Genetics Database, which is supported by the Specialized Cooperative Centers Program in Reproduction Research (SCCPRR).

Y-Chromosome Sequence and Evolution

The Y chromosome is the male-determining chromosome that houses genes critical for normal male development and fertility. The highly repetitive nature of Y-chromosome DNA originally frustrated sequencing efforts; however, a team of RSB-funded scientists successfully sequenced the male-specific region of the Y chromosome. They found three large blocks of heterochromatin (highly condensed, inactive DNA) interspersed between the euchromatin (active DNA), which they estimate encodes 27 proteins, half of which are testes-specific. The euchromatin consists of DNA recently transposed from the X chromosome, remnants of ancient autosomal DNA, and extraordinary ampliconic sequences (e.g., large repeat regions) with as much as 99.9 percent similarity to each other. The amplicons are the most gene-rich regions of the Y chromosome, and the genes are mainly testes-specific. The presence of such huge, precise repeats that code for testes-specific genes is not consistent with the view of the Y chromosome as a disintegrating and degenerate chromosome. Instead, these regions might maintain themselves through gene transfer, meaning the repeats recombine with each other. The internal recombination, while beneficial to the Y chromosome as an entity, might increase the risk of gene deletions that cause infertility.

DEVELOPMENTAL BIOLOGY OF REPRODUCTION

Developmental biology of reproduction encompasses fertilization, preimplantation embryo development, implantation, sex determination, and establishment of the germ line. Together, these processes culminate in the formation of a new individual capable of continuing the generative or reproductive cycle. This portion of the Branch's portfolio also includes studies on the derivation, self-renewal, and differentiation of embryonic stem cells (ESCs) and embryonic germ cells.

Germ Plasm Proteins in the Designation of Germ Line versus Somatic Cells

Unequal cell divisions in the egg of the worm *Caenorhabditis elegans* establish the germ cells as a separate population from the somatic cells. At least five zinc-finger proteins, which are normally found in the germ plasm, are targeted for selective degradation in somatic cells by a specific RNA-binding protein and other associated proteins. New evidence suggests that the separation of the germ-cell and somatic-cell lineages is accomplished by depriving the somatic cells of key proteins, rather than by enriching the germ cells with them. Mutant embryos die if they lack the specific RNA-binding proteins because the zinc-finger proteins persist—a situation that is incompatible with somatic-cell development. In addition, other proteins in *C. elegans* eggs selectively protect these same zinc-finger proteins from degradation in the germ plasm, a key part of establishing germ-line cells as separate from somatic cells. The overall effect of this selective degradation and protection of specific molecules is that key germ plasm components are restricted to the germ-plasm lineage, allowing normal germ-cell development in the early embryo. Detailed information about early decision-making processes in embryonic development and establishment of the germ line can provide novel insights into the unknown mechanisms that control the same processes in humans.

Long-Term Effects of *In Vitro* Embryo Culture

There are now approximately three million children world-wide born as a result of *in vitro* fertilization (IVF); however, few studies of the long-term outcomes of these children have been conducted (Adamson *et al.*, *Fertility and Sterility*, 85, 1586-1622, 2006). For more than 40 years, preimplantation embryo culture has proceeded successfully even though the culture media for human embryos are inferior to the natural environment of the oviduct and uterus. New results indicate subtle, long-term behavioral deficits in mice that developed in inferior culture media during the preimplantation embryo stage, as compared with those grown in superior culture media. The mice raised in inferior culture media showed significant deficits in tests of anxiety, locomotor activity, and spatial memory. In addition, the inferior culture media "erased" imprints at the H19 gene, suggesting a correlation between embryo culture, early epigenetic damage, and abnormal subsequent adult behavior. As part of the NICHD Cooperative Program on Female Health and Egg Quality, a new study is using microarray analysis to investigate how the environment of the mouse embryo—*in vivo*, in culture *in vitro* with inferior medium, or in culture *in vitro* in superior medium—affects the pattern of global gene expression. This research might also provide insights into the mechanisms of how inferior culture media impact both embryo development and adult behavior.

NPM2 is Critical for Nuclear and Nucleolar Organization and Preimplantation Development

Shortly after fertilization, crucial factors from the egg carry out the nuclear and nucleolar remodeling essential for the formation of a diploid, zygotic genome. In *Xenopus,* one such factor, nucleoplasmin 2 (*Npm2*), promotes the reorganization of the paternal DNA. An RSB-funded laboratory identified the homolog of *Npm2* in mice, the first time such a molecule has been found in mammals. Although male mice that lacked the *Npm2* gene were normal and fertile, the females were subfertile because their fertilized eggs could not complete the transition to the two-cell stage. In mice, *Npm2* is involved in the normal decondensation and reorganization of the maternal DNA of fertilized eggs, but has minimal effect upon the paternal DNA. A similar transitional block from the one-cell to two-cell stage occurs in mice that lack the *Zar1* gene because the maternal and paternal genomes fail to combine. Therefore, *Zar1* seems to act earlier in embryonic development than does *Npm2*. As yet, *Npm2* and *Zar1* are the only oocyte-specific proteins known to be essential for development from the one-cell to the two-cell stage. Future studies will help reveal how *Zar1* and *Npm2* function in early development, whether or not they are present in human eggs and early embryos, whether their mutation might cause some types of infertility in women, and whether they could serve as novel contraceptive targets.

Unique Patterns of Gene Expression among Human Embryonic Stem Cell (hESC) Lines

Each hESC line is unique because it is derived from one unique blastocyst. It would follow, then, that gene-expression profiles for each human blastocyst would differ genetically because of parentage, and that the profiles would also differ epigenetically owing to aspects of the IVF protocol. Researchers recently published the first study of the degree of similarity in gene expression between different hESC lines, comparing the developmental capacity and gene-expression profiles of three hESC lines. All cell lines differentiated *in vitro* into the three tissue layers (namely, endoderm, ectoderm, and mesoderm), but the differentiation time course varied significantly between the three lines. The expression of many genes was limited to just one or two cell lines. Microarray analysis also demonstrated that only 52 percent (7,385) of the genes investigated were commonly expressed in all three cell lines; the levels of expression of these genes were also significantly different among the cell lines. Thus, individual hESC lines do have unique gene-expression signatures and express the genes necessary for self-renewal and for the differentiation of multiple cell types from these hESCs.

Embryonic Stem Cells (ESCs) can Develop into Germ Cells *In Vitro*

When injected into a blastocyst, true ESCs can develop into all somatic cell types as well as into germ-line cells, the precursor cells of sperm and eggs. However, no researchers had reported ESCs developing into germ cells *in vitro*. Recently, however, a laboratory showed that mouse ESCs *in vitro* can develop into egg-like cells as well as into blastocyst-like structures.

Another RSB-supported study showed that hESCs can also begin germ-cell formation *in vitro,* although meiosis was not completed. The inner cell mass and hESCs express several markers of pluripotent cells, but do so in addition to the hESC-expressed DAZL, a marker of the pre-meiotic germ-cell program. The presence of DAZL showed that immature germ cells were formed from the hESCs *in vitro*. When the hESCs were coaxed into developing into embryoid bodies *in vitro,* they expressed six molecular markers of meiosis in the putative germ cells.

In the long term, such breakthroughs might be useful in animal breeding, in human ART, and for delving into the origins of germ-cell tumors. Importantly, these advances also provide novel insights into the normal processes by which undifferentiated cells develop into primordial germ cells, and how primordial germ cells develop into mature sperm and egg cells.

Oocyte Genes that Affect Cloning and Nuclear Reprogramming

Oocyte genes and/or proteins control the reconstruction of the sperm nucleus when it enters the egg. Likewise, during cloning, some form of egg control is exerted upon ESC nuclei and on adult somatic-cell nuclei that are transplanted into enucleated eggs. Recent experiments tested whether the same genetic system controls the egg's response to both sperm pronuclei and to transplanted nuclei. Because efficiency of natural and assisted reproduction varies among mouse strains, researchers assessed the egg response to transplanted nuclei in various types of mice. The studies used parthenogenesis and somatic-cell nuclear transfer to eliminate variations introduced by the paternal genome and sperm factors. Both test methods showed strain-specific differences in success rates, suggesting that particular oocyte genes dictate a strain-specific phenotype, which exerts control of any nucleus entering the oocyte. Thus, it is quite possible that the same genes dictate success or failure of normal reproduction and the success or failure of cloning and nuclear reprogramming. Regulating critical genetic and epigenetic factors might improve the success rate of cloning and minimize developmental abnormalities in cloned embryos.

First Successful Cloning of Terminally Differentiated, Post-Mitotic Cells in Mice

The advent of cloning by transferring an adult somatic cell into an egg seriously challenged the dogma that development from egg to complex organism involved irreversible changes in the chromosomes. When injected into enucleated mouse eggs, genetically marked cells from the olfactory epithelium of adult mice were reprogrammed to direct the development of an entire organism, including the germ cells. The adult olfactory cells also gave rise to ESCs, which could be reprogrammed to differentiate into any cell of an organism. Thus, the original precise specification of the selected odorant receptor cell is reversible: That is, through cloning, the differentiated cells reverted to a totipotent state. This finding provides the first evidence that, when injected into oocytes, post-mitotic terminally differentiated cells can be reprogrammed. The work not only opens the door to experiments with other terminally differentiated cells, but also might provide insights into how to directly reprogram terminally differentiated adult cells into other types of adult cells for transplantation without the need for therapeutic cloning.

Role of Germ Cells in Sex Determination

Abnormalities of sexual differentiation are common and can range in severity from abnormal looking genitalia (1 in 100 newborns) to complete sex reversal, in which the gonads and the sex chromosome composition are discordant (1 in 20,000) (Fleming A & Vilain E, *Clinical Genetics,* 67, 15-25, 2004). Therefore, understanding the precise mechanisms of sex determination in humans is important. Even though normal testes can develop in the absence of germ cells, germ cells are essential for the development of an ovary. A series of studies conducted by an RSB-funded researcher found that during a critical period, prior to the intrinsic onset of meiosis, cultured mouse female gonads can be "tricked" into developing into testes. Meiotic germ cells are normally found only in female embryonic gonads because, in normal male embryos, the germ cells are sequestered in testes cords before they can enter meiosis. The presence of meiotic germ

cells dictates the commitment to the female pathway, meaning that the meiotic germ cells themselves, not an intrinsic gonadal "default" clock, prompt ovarian development. Female meiotic germ cells are not only pro-ovary, but also anti-testes and disrupt testes cord formation in cultured male gonadal tissue. Female somatic cells, even those from older embryos, do not have this anti-testes action. These data suggest that the presence of meiotic germ cells promotes the ovarian fate and inhibits testes development, meaning that female development is an active process and not merely a "default" pathway.

Development of the Reproductive Tract

During normal male embryonic development, the testes secrete Anti-Mullerian hormone (AMH), which triggers degeneration of the Mullerian ducts that would become the oviducts, uterus, and vagina in a female. Researchers had previously identified the type II receptor for AMH, *Amrh2*, but the type I receptor was unknown. RSB-supported investigators used a gene deletion system that was dependent on the expression of *Amrh2* to specifically inactivate candidate genes in the developing Mullerian ducts. When they eliminated the gene for type 1 bone morphogenetic protein receptor-1a (*Bmpr1a*, also known as *Alk3*), male embryos retained the Mullerian ducts and developed oviducts and uteri, indicating that *Bmpr1a* is the type 1 receptor for anti-Mullerian hormone.

As explained earlier, errors in sex determination are quite common in humans, and this particular error—retention of Mullerian ducts—causes infertility in affected men. Identifying *Bmpr1a* as the type 1 AMH receptor advances the understanding of exactly when and how the critical remodeling of the reproductive ducts occurs. In addition, the development of the *Amrh2* conditional deletion system provides a valuable resource for other investigators who are studying the early development of the gonads and reproductive tract.

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 facilities. The SCCPRR is funded through the U54 cooperative mechanism, which provides support for research projects and core services. Center investigators work with NICHD staff to facilitate research collaborations within and between centers. Locations of the 13 SCCPRR sites appear in Figure 1.

To promote interactions among centers, the SCCPRR established research focus groups that included investigators from different centers focused on the areas of male reproduction, endometrial biology, ovarian physiology, and neuroendocrinology. These groups meet twice each year to exchange information and discuss potential collaborations. Collaborating investigators may submit Collaborative Research Initiatives (CRIs) on topics related to their

SCCPRR research. These initiatives require the expertise of several centers and usually have a clinical/applied focus. Overall, the Program has supported five CRIs; three of these initiatives were co-funded by the NIH ORWH.

An additional focus group monitors the technology/informatic needs of the SCCPRR and the reproductive sciences community at-large. Among the technology services that have resulted from this focus group are:

- The **Population Center Gene Array Facility** at the University of Washington performs gene expression profiling for the SCCPRR;
- The **National Center for SCCPRR Proteomics** at the University of North Carolina offers state-of-the-art proteomic services to SCCPRR investigators; and
- The University of Virginia's Center for Research on Reproduction provides SCCPRR-wide access to their **Ligand Assay and Analysis Core Facility**.

The SCCPRR also supports tissue banks for human endometrium, human ovary, male reproductive tissues and fluids, and non-human primate tissues. Access to these banks is open to North American investigators who are supported by research and research training grants from the NIH. Investigators can make online tissue requests at the **Reproductive Tissue Sample Repository (RTSaR)**. Visit https://rtsar.nihcd.nih.gov/rtsar/login to log into the database.

The SCCPRR provides funding for online gene/protein databases, including the:

- Ovarian Kaleidoscope Database (http://ovary.stanford.edu/)
- Endometrial Database Resource (http://endometrium.bcm.tmc.edu/edr/)
- Mammalian Reproductive Genetics Database (http://mouse.genetics.washington.edu/)
- Reproductive Tissue Gene Expression Database (http://intermedin.stanford.edu/db/RTGED.htm)

The following examples highlight multi-center collaborations initiated by the focus group meetings.

Identification of a Novel Puberty Gene Provides Insight into GnRH Regulation

The control of sexual maturation remains one of the great mysteries of human biology. As puberty begins, the GnRH system is activated, stimulating the production of gametes and secretion of sex steroids. Investigators at the Harvard University SCCPRR site found that some patients afflicted with idiopathic hypogonadotropic hypogonadism (IHH), defined as lack of puberty, have mutations in the gene that encodes a G protein-coupled receptor, *GPR54*, which regulates GnRH. Mice engineered to lack the *Gpr54* gene also failed to reach puberty even though they had normal amounts of GnRH in the hypothalamus. Additional work by investigators from the University of Washington, Oregon Health and Science University, and the University of Pittsburgh SCCPRR sites demonstrated that administration of kisspeptin, the endogenous ligand for *GPR54*, stimulated release of LH in lab animals, an effect that could be blocked by pretreatment with a GnRH antagonist. These collaborative studies demonstrate that mutations in *GPR54* can cause IHH in both humans and mice, and that kisspeptin binds *GPR54* to regulate GnRH secretion.

Molecular Phenotyping of Endometrium

Endometriosis, a disorder characterized by ectopic uterine growths, pelvic pain, and infertility, afflicts 10 percent to 15 percent of reproductive-aged women. SCCPRR investigators surveyed endometrial gene expression during the window of implantation and analyzed 12,686 genes. Results revealed that expression of 91 genes was significantly increased, while expression of 115 genes was significantly decreased in the endometrium of women who had endometriosis compared to controls. Women with endometriosis had decreased endometrial expression of the gene that encodes an enzyme for synthesizing L-selectin, a molecule that helps the embryo attach to the uterine wall. These data support the concept that dysregulation of certain genes causes endometriosis. Other genes identified by expression profiling might provide targets for novel diagnostic and intervention strategies for endometriosis.

Early Ovarian Aging in Carriers of the Fragile X Syndrome Premutation

About 16 percent of women who have the Fragile X Syndrome premutation (defined as those with between 55 and 200 repeats in the promoter region of the Fragile X Mental Retardation 1 or *FMR1* gene) are at risk for premature ovarian failure (POF), defined as the end of menstrual cycles before the age of 40. Investigators supported by several SCCPRR sites, the National Cooperative Program for Infertility Research, the NICHD Division of Intramural Research, and Emory University found that carriers of the Fragile X premutation had overt hormonal signs of early reproductive aging (e.g., elevated circulating FSH levels, low inhibin B levels) despite normal menstrual cyclicity. This information could be useful in fertility counseling for women carrying the Fragile X premutation and their families.

Evidence of Ethnic Differences in Adolescent Girls with PCOS

PCOS affects approximately 4 million reproductive-aged women, and costs for evaluating and providing care to these women exceeds $4 billion annually (Azziz *et al.*, *Journal of Clinical Endocrinology and Metabolism*, 90, 4650-4658, 2005). The disorder is defined by anovulation and high androgen levels, and many patients also present with obesity and cystic ovaries. Another hallmark of the syndrome, elevated plasma levels of LH, might result from a dampened response to negative feedback suppression by progesterone. Investigators from SCCPRR sites at the University of Virginia and University of California, San Diego, studied the ability of progesterone to suppress circulating LH levels in nine normal control girls and in 11 adolescent girls with hyperandrogenemia, a harbinger of full-blown PCOS. Progesterone suppressed LH levels in all normal control girls, and in the five hyperandrogenemic Hispanic girls. However, progesterone failed to suppress LH in the other six hyperandrogenemic girls (four Caucasians and two African Americans). PCOS and adolescent hyperandrogenemia are associated with altered responsiveness to progesterone, and the ethnic differences in this response suggest a possible genetic component.

Live Birth from a Three-Dimensional Ovarian Culture System

Complex intercellular interactions in the ovary have made *in vitro* follicle development a very difficult and inefficient endeavor, greatly limiting the options for women whose fertility is threatened by cancer treatments. Now, SCCPRR investigators at Northwestern University have developed an innovative three-dimensional culture system for ovarian follicles that maintains cell interactions *in vitro*. They recently established proof-of-principle for *in vitro* development of human follicles when they reported the live births of mice from this culture system. If

successful, this procedure could make fertility preservation a reality for women who have cancer and need to undergo radiation or chemical treatments.

Visit http://reprobio.stanford.edu/sccprrnet/ for more information about the SCCPRR.

NATIONAL COOPERATIVE PROGRAM FOR INFERTILITY RESEARCH (NCPIR)

The NCPIR was formed in 1991 in response to a congressional recommendation for infertility research centers that could advance development of new diagnostics, therapeutics, and cures for the detection, management, and alleviation of human infertility. The Program uses the U54 center mechanism to fund efforts at the University of Pennsylvania, which has a consortium project at Northwestern University, and at Massachusetts General Hospital, in collaboration with deCode Genetics (based in Iceland).

A primary focus of the NCPIR is understanding the genetic basis of PCOS, a condition that affects 5 percent to 10 percent of reproductive-aged women and the leading cause of anovulatory infertility. Hyperandrogenemia, due to excessive androgen secretion by ovarian theca cells, is a hallmark of PCOS. Using gene-expression profiles, NCPIR investigators found that theca cells from women with PCOS have a distinct molecular signature that includes increased expression of GATA6, a transcription factor that stimulates the expression of the P450 17α-hydroxylase (*CYP17*) gene; increased GATA6 promotes androgen synthesis. Additionally, the half-life of the *CYP17* gene is longer in PCOS theca cells than in normal theca cells. NCPIR research also identified a region on chromosome 19p13 that contains genetic determinants for PCOS.

NCPIR research results also show a higher prevalence of the metabolic syndrome (e.g., cardiovascular risk factors associated with insulin resistance) in adolescents with PCOS than in the general adolescent population. These findings also indicate that hyperandrogenemia, independent of obesity and insulin resistance, increases the risk of metabolic syndrome. Early identification of the metabolic syndrome in adolescents with PCOS and appropriate intervention could reduce the long-term health risks of diabetes and cardiovascular disease in these patients.

NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK (RMN)

The RMN was established in 1987 to conduct large multi-center clinical trials on the treatment of female and male infertility and reproductive diseases and disorders. The eight clinical sites in the RMN appear in Figure 2.

The Data Coordinating Center (DCC), at Duke University, maintains an RMN Web site with secure access for RMN investigators as well as public access (visit http://rmn.dcri.duke.edu for more information).

The RMN operates with a Steering Committee, an independent Clinical Trials Advisory Board, and a Data Safety and Monitoring Committee. In 2002, the RMN transitioned to a new budget process that included a base for each center's fixed costs and a capitated budget for each protocol.

RMN research has yielded numerous results that have advanced the scientific understanding of infertility. For example, one study showed that timed endometrial biopsy was not a helpful screening tool for infertility. More recently, the RMN conducted a randomized trial comparing the effects of clomiphene, metformin, and combination metformin and clomiphene for treating infertility in women with PCOS. The study showed that, contrary to expectations, clomiphene alone was superior to metformin, an insulin-sensitizing agent, for induction of ovulation, conception, and pregnancy.

COOPERATIVE PROGRAM ON TROPHOBLAST-MATERNAL TISSUE INTERACTIONS

Scientists in this cooperative program, which includes seven U.S. institutions, investigate the roles of selected molecules at the interface between trophoblast and maternal tissues during early pregnancy. This research has clinical relevance to pregnancy failure, ectopic pregnancy, and abnormal placentation associated with preeclampsia. Several important findings have emerged from this research. For instance, new data show that interactions of L-selectin with its carbohydrate receptors play an important role in human implantation, and that glycosylation of the receptors is hormonally regulated. The uterine glycoprotein MUC1, a critical inhibitor of embryo attachment, is controlled by removal at the cell surface (shedding) as well as at the level of gene expression by cytokine-activated transcription factors and by progesterone receptors. Another project found that embryonic trophoblast cells stimulate expression of tight junctional proteins in the uterine decidual cells that immediately surround the embryo. This embryo-induced decidual zone prevents the passage of maternal immunoglobulins and immune cells to the embryo, shielding the embryo from potential harm.

NATIONAL COOPERATIVE PROGRAM ON FEMALE HEALTH AND EGG QUALITY

At or near the time of fertilization, even briefly exposing mammalian eggs to poor maternal nutrition or other adverse conditions, including some ART procedures, has long-term effects on offspring derived from these eggs. These long-term effects might include preterm birth, low birth weight, hypertension, abnormal organ growth, and abnormal adult behavior. Thus, the RSB established the National Cooperative Program on Female Health and Egg Quality to support an international team of scientists in studying the long-term effects of pre- and peri-conceptual insults to eggs and early embryos in several animal models and in human eggs. With improved understanding of the nature and actions of these insults, perhaps researchers can identify measures to improve egg and embryo quality in both natural and assisted reproduction so as to avoid or minimize harmful long-term effects.

COOPERATIVE PROGRAM FOR MOUSE PHENOTYPING: DEVELOPMENTAL AND FERTILITY DEFECTS

This cooperative program began in the fall of 2002, in an effort to phenotype and map mutations that affect development and fertility in mutant mice created in an earlier trans-NIH project. The program includes investigators at Baylor College of Medicine, Northwestern University, Harvard University, and Sloan-Kettering Institute and benefits from the informal cooperation of Dr. Monica Justice (Baylor College of Medicine) and Dr. John Eppig (Jackson Laboratory). The group coordinates its activities with the NIH Mouse Genomics and Genetics Scientific Panel.

The Baylor and Northwestern projects focus on fertility mutants. The Baylor program optimized the "sleeping beauty" transposon to rapidly create transgenic mutants and efficiently clone the affected gene. These researchers are now characterizing several mutant phenotypes. The transgenic mutagenesis program at Baylor has generated six confirmed and four probable reproductive mutants. The Northwestern project is screening for dominant testicular phenotypes and has identified 36 mutant lines, three of which are confirmed. One mutant is reminiscent of the human syndrome Congenital Anomalies of the Kidney and Urinary Tract (CAKUT). All described mutants are freely available to the research community through the projects' Web sites; in addition, many mutants will be archived as frozen sperm as part of the Mutant Mouse Regional Resource Centers.

RSB CO-FUNDED RESEARCH PROGRAMS

The RSB routinely cooperates with other NIH entities to further common research goals. Because the genitourinary tract is the most common site of birth defects, including hypospadias and cryptorchidism, the National Institute of Diabetes and Digestive and Kidney Diseases and the NICHD began the Genitourinary Developmental Mouse Atlas Project cooperative program in 2004. The program will develop a high-resolution gene-expression atlas and anatomical atlas of the developing murine genitourinary tract to produce an integrated database and other reagents (such as mouse lines, antibodies, and protocols) for the entire research community.

In December 2001, the NICHD, six other NIH Institutes, and the Food and Drug Administration joined the ORWH in initiating the Specialized Centers of Research (SCOR) on Sex and Gender Factors Affecting Women's Health Program to foster interdisciplinary research and improved approaches for the treatment and/or prevention of human disease. The three centers co-sponsored by the NICHD use a combination of basic and clinical research projects to study maternal and fetal drug interactions, PCOS, and pelvic floor dysfunction.

Through the RSB, the NICHD has joined a number of NIH Institutes and centers in co-funding various initiatives in the area of stem cells. One such initiative, active since 2002, has provided funding to support T15 training courses in hESC culture techniques. These courses have trained approximately 100 new researchers per year at several key centers in the maintenance, characterization, and utilization of hESCs. The RSB has also provided support for the hESC Research Resource Infrastructure Enhancement Award, which uses the R24 mechanism, to

increase the availability of hESC lines for research through the expansion, testing and characterization, quality control, cryopreservation, and distribution of NIH-approved hESC lines.

RESEARCH TRAINING PROGRAMS AND CAREER DEVELOPMENT

INDIVIDUAL TRAINING PROGRAMS AND CAREER DEVELOPMENT AWARDS

The RSB supports the development of highly skilled reproductive science investigators through individual postdoctoral fellowships and career development awards to funded scientists and clinicians. Individual postdoctoral fellowships (F32s) are awarded to newly trained scientists, for up to three years. The award enables them to work full time with a qualified mentor and to develop expertise in an eligible field of reproductive science research (see Table 2).

Scientists who have outstanding research potential receive the individual career awards (K series) to release them from teaching and administrative responsibilities and to allow a period of intensive focus on research (see Table 3):

- Independent Scientist Award (K02) is used to promote the careers of newly independent scientists.
- The Mentored Clinical Scientist Development Award (K08), the Mentored Patient-Oriented Research Career Development Award (K23), and the Mid-Career Investigator Award in Patient-Oriented Research (K24) provide protected time for clinicians to conduct research.
- From 2000-2005, the RSB also used the K25 Mentored Quantitative Research Career Development Award to support a fellow with engineering or quantitative science background, who was interested in pursuing biomedical research.

INSTITUTIONAL TRAINING PROGRAMS AND CAREER DEVELOPMENT AWARDS

Institutional training grants (T32s) are awarded to outstanding educators at leading U.S. institutions, enabling them to establish and maintain an appropriate environment for reproductive sciences research training (see Table 4). The T32 training sites supported by the RSB are shown in Figure 4. Two Principal Investigators (PIs) from the T32 program have received NICHD MENTOR awards for outstanding training programs.

Reproductive Scientist Development Program (RSDP)

The RSDP is a multidisciplinary, multi-institutional research career development program for obstetrician-gynecologists studying cellular and/molecular biology and genetics and related fundamental sciences. This Program uses the NIH Mentored Research Scientist Development Program Award (K12) mechanism. Under the leadership of Dr. Robert Jaffe at the University of California, San Francisco, the RSDP is now in its 18th year.

Collaborative funding from private sources is an integral and unique part of the Program's design—it is sponsored jointly by the NICHD, four professional organizations, four pharmaceutical corporations, and four research foundations. In Phase I, scholars spend two or three years in mentored intensive training in basic science. In Phase II, scholars spend an additional three-year period establishing their research programs as junior faculty in a department of obstetrics and gynecology. Since the RSDP began in July 1988, the program has trained 60 obstetrician-gynecologist physician-scientists, many of whom went on to establish highly successful careers in academic medicine.

Recent work by RSDP scholars has provided new insight into ovarian cancer. In one study, investigators found a correlation between stress and levels of vascular endothelial growth factor, a marker of stress and a key player in blood vessel formation in tumors. In another study, microarray analysis of ovarian cancer cells treated with the drug epothilone B demonstrated that the drug altered the expression of 41 genes in the tumor necrosis factor-alpha pathway, arrested the cell cycle, and caused apoptosis (defined as programmed cell death). New drugs to treat ovarian cancer are a high priority because the regimens currently in use result in a 50-percent relapse rate within two years, and because patients frequently develop resistance to the drugs. These findings offer novel targets for therapy regimens.

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

The NICHD initiated the WRHR Career Development Program in 1998 to increase the number of obstetrician-gynecologist physician-scientists performing research on women's health. The NIH ORWH and the National Cancer Institute collaborate with the NICHD to support this K12 Program, which bridges clinical training with an independent career in research to address women's health. Investigators in established research programs, which cover a broad range of basic and applied biomedical and biobehavioral science, and in obstetrics and gynecology departments and collaborating departments form an intellectual and technical research base for mentoring WRHR scholars who are junior faculty members. The Program's emphasis is on research relevant to obstetrics and gynecology and/or its subspecialties, including: maternal-fetal medicine, gynecologic oncology, and reproductive endocrinology and infertility. Related fields, such as adolescent gynecology and urogynecology, are also included.

There are 20 active WRHR Centers, and to date, 106 scholars have been appointed to faculty positions. These scholars comprise a diverse group of physician-scientists from several subspecialty and emerging areas in obstetrics and gynecology who are pursuing a broad range of basic science, translational, and clinical research topics. Locations of the 20 WRHR Programs appear in Figure 3. Visit http://www.wrhrscholars.org for more information about the WRHR Program.

Male Reproductive Health Research (MRHR) Career Development Program

Modeled after the WRHR Career Development Program, the MRHR Career Development Program was initiated in the fall of 2006 with the intent of fostering the mentored career development of clinicians who specialize in male reproductive health. The goal of this effort is to increase the clinical research capacity among practitioners in the area of male reproductive biology and to promote the translation of basic science advances to clinical practice. Awards

were made to two institutions: the University of Washington, and the University of California, San Francisco.

Building Interdisciplinary Research Careers in Women's Health (BIRCWH) Career Development Program

In 2000, the NIH ORWH established the BIRCWH Program as an institutional research career development award for junior faculty members who recently completed clinical training or postdoctoral fellowships, and who were pursuing basic, translational, clinical, and/or health services research relevant to women's health. This K12 Program aims to promote research on and the transfer of findings relevant to women's health, including sex/gender similarities or differences in biology, health, or disease, through participation in mentored research and career development activities, with the end result of an independent interdisciplinary scientific career addressing women's health. The ORWH, along with the NICHD, several NIH Institutes and Offices, and the Agency for Healthcare Research and Quality, have supported a total of 287 BIRCWH scholars since the fall of 2000. Through the RSB, the NICHD administers the BIRCWH Program on behalf of the ORWH.

INTERNATIONAL TRAINING PROGRAMS

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. Through the Americas Fellowship Program, which is co-funded by the NICHD and the NIH Fogarty International Center through a supplement to the D43 mechanism for International Training and Research in Population and Health, postdoctoral investigators from Latin American member countries can train at leading North American institutions for up to 24 months. To date, the RSANET Project has trained 10 fellows. In the future, the Americas Fellowship Program will use the F05 International Research Fellowship Award to support foreign fellows who work in the United States. Monthly electronic reports enhance communication among the investigators and announce conferences and research opportunities.

Frontiers in Reproduction (FIR) Course

The RSB has played a leading role in launching and maintaining this international training course in techniques and concepts of advanced reproductive biology research since the course debuted in 1998 at the Marine Biological Laboratory, Woods Hole, Massachusetts. Supported jointly by the NICHD, the Burroughs Wellcome Fund, the United Nations Educational, Scientific, and Cultural Organization (UNESCO), and other professional and private sources, the FIR course provides an intense six-week learning experience to 16 postdoctoral or junior faculty scholars. A combination of laboratory work, lectures, and symposia gathers an international faculty and attracts applicants from as many as 23 countries. In 2001, the course and symposium were brought together under the multi-year T15 training mechanism.

TRAINING OPPORTUNITIES FOR MINORITIES AND MINORITY INSTITUTIONS

Specialized Cooperative Reproductive Science Research Centers at Minority Institutions Program

This Program aims to strengthen the research capabilities of faculty, students, and fellows at minority institutions. The NICHD formed this cooperative Program along with the NIH ORWH, the National Center for Research Resources, and the National Center for Minority Health and Health Disparities (NCMHD). Participating investigators at minority institutions create collaborative partnerships with established NICHD-sponsored institutional programs to address priority areas of reproductive science research. Current sites include: Morehouse School of Medicine; Meharry Medical College; and Charles R. Drew University of Medicine and Science. These Centers bring together strong teams of investigators to focus on a reproductive science topic, and to share essential facilities, services, knowledge, and other resources.

Programs to Support Research by Minorities and Persons with Disabilities

The RSB uses several tools to increase the participation of underrepresented groups in research and research training. In terms of training grants, the Institute maintains a firm policy that institutional training programs propose and carry out a plan for recruitment of minorities that is rigorously monitored for all new, competing, and non-competing applications. In addition, F31 pre-doctoral fellowships support eligible candidates from minority groups and/or those with disabilities. An NIH-wide supplement program is also available to PIs who are mentoring minority or disabled trainees from high school level through the new investigator level. In November 2004, the NIH transitioned these Programs to the *Research Supplements to Promote Diversity in Health-Related Research* effort, which broadened eligibility to include minority, disabled, and/or disadvantaged individuals (visit http://grants1.nih.gov/grants/guide/pa-files/PA-05-015.html for more information). Through this new Program, the RSB has supported 6, 14, 17, and 13 individuals in fiscal years 2003, 2004, 2005, and 2006, respectively.

PORTFOLIO ANALYSIS AND PROGRAM EVALUATION

During the past four years, the Branch embarked on an extensive analysis of its eight program areas to provide Branch staff with information to better manage their portfolios in terms of program balance (i.e., number of grants and dollars spent in a particular science area), and to more effectively identify under-researched areas that may be in need of expansion. Initially, the Branch divided each portfolio into specific research areas (see Table 1) and coded grant applications submitted for fiscal year 2001 through fiscal year 2006. The distribution of dollars and number of projects and for each subcategory for the duration of these fiscal years appears in Figure 13A and Figure 13B and Figure 14A and Figure 14B, respectively. Figure 15A, Figure 15B, Figure 16A, Figure 16B, Figure 17A, Figure 17B, Figure 18A, Figure 18B, Figure 19A, Figure 19B, Figure 20A, and Figure 20B depict the success rates for all grants submitted within a given portfolio and for some specific science areas within those portfolios. The success rates for each portfolio varied from 22 percent to 28 percent during this timeframe.

The plight of new investigators in obtaining research support is of critical importance to the NIH, especially during times of budget constraints. Accordingly, the RSB has made special efforts to maximize the number of new investigators receiving research support. From fiscal year 2003 to fiscal year 2006, the success rate of new investigators in obtaining grant support through the R01, R21, and R03 mechanisms trended higher for RSB-assigned applications than for applications assigned to the rest of NICHD (Figure 23 and Figure 24). Within the cohort of RSB-assigned applications from new investigators, the success rate for garnering R03 support was higher than for obtaining R01 support in four of seven years between fiscal year 2000 and fiscal year 2006 (Figure 25).

As expected, the success rate of established investigators in obtaining R01 support was higher than the success rate for new investigators in all but fiscal year 2000 (Figure 30). Interestingly, for fiscal years 2000 to 2002, the success rate of new investigators in obtaining R03 support was higher than the success rate of R03-seeking established investigators; the opposite was true for fiscal year 2003 through fiscal year 2006, during which time established investigators were more successful (Figure 31).

The Branch also sought to determine the impact that the reorganization of CSR study sections had on the success rate of applications assigned to the RSB. This information would be helpful for staff to use in answering questions from potential grantees about which study section would be best suited to review a particular science area. Success rates for the new study sections were determined for applications assigned to the RSB in fiscal year 2005 and fiscal year 2006 and were compared with the success rates of all applications submitted to those study sections. In addition, Branch staff compared success rates of the new study sections (fiscal year 2005 and fiscal year 2006) and the previous study sections, present prior to the reorganization (fiscal year 2001 through fiscal year 2004); fiscal year 2004 data was separated from fiscal years 2001, 2002, and 2003 because the NICHD percentile payline was markedly lower in 2004 than in the previous three fiscal years. In general, for fiscal year 2005 and fiscal year 2006, the success rate for applications assigned to the RSB was comparable to the success rate for all applications reviewed by the Cellular, Molecular, and Integrative Reproduction (CMIR) Study Section (Figure 32) and by the Integrative and Clinical Endocrinology and Reproduction (ICER) Study Section (Figure 33), two study sections that review large numbers of RSB-assigned applications. The success rate of RSB-assigned applications reviewed in the Reproductive Biology (REB) Study Section (the predecessor of CMIR) and the Reproductive Endocrinology (REN) Study Section (the predecessor of ICER) was comparable to the success rate of all applications assigned to those two study sections between fiscal year 2001 and fiscal year 2004.

Figure 34 shows that, for fiscal years 2005 and 2006, the success rate for RSB-assigned applications was comparable to the success rate of all applications reviewed in the Pregnancy and Neonatology (PN) study section. In contrast, the RSB-assigned application success rate was markedly lower than the success rate of all applications reviewed in the Development 1 and Development 2 Study Sections. Prior to the reorganization, many of the grants assigned to these new study sections were reviewed in the Human Embryology and Development-1 Study Section, for which the success rate of RSB-assigned applications was slightly lower than the overall success rate for fiscal year 2001 through fiscal year 2003.

Branch staff also actively evaluated several of the Branch's special programs, including the NCPIR and the RMN, which underwent extensive formal evaluations. As a result of these evaluations, the Branch will merge the NCPIR with the SCCPRR initiative and will restructure the RMN. The Branch also conducted a less extensive mid-course evaluation of the SCCPRR; the recommendations from that evaluation were incorporated into the annual request for application. The WRHR Career Development Program also underwent a feasibility study in preparation for a formal evaluation, which will occur during the next few years.

FUTURE DIRECTIONS FOR THE BRANCH

To increase accountability and transparency in the strategic planning process for the Branch, the RSB sought expertise and feedback on its future directions from an expert panel, which consisted of scientists in the fields of reproductive biology and medicine, representatives from scientific and advocacy groups, and two liaisons to the NACHHD Council. (See Appendix F for a list of panel members.) Panel members received a multitude of resources before the meeting to acquaint them with the mission and funding history of the RSB. Like the expert panels assembled by other Branches, the RSB panel was charged with addressing three overarching questions as a springboard for discussion, including:

- Given its mission, what are the most important scientific opportunities that the Branch should try to pursue in the next four years?
- Given its mission, what are the most important public health issues that need to be addressed by the Branch over the next four years?
- What areas deserve less emphasis because progress has been made or will continue without further stimulation from the NICHD in general and the RSB in particular?

The panel met with Branch staff for two days to discuss the Branch's performance and to make recommendations concerning scientific opportunities and public health issues the RSB could pursue in the next four years, as well as scientific areas that the Branch might de-emphasize because they no longer needed NICHD stimulation to advance.

PANEL DISCUSSION

The panel commended the RSB for successfully leveraging dollars and for using resources efficiently. The panel especially appreciated Branch staff's own internal system of tracking applications by specific scientific area within a given program portfolio (see Table 1). The system allows Branch staff to identify emerging areas of science that are building momentum; science areas that are deemed critical, but need stimulation; and success rates of applications for various study sections. The latter is important for helping applicants determine which review groups may be best suited to review their proposals.

One overarching concern noted by the panel was that, although RSB supports scientifically exciting and significant research, this sense of excitement was not fully appreciated in the general scientific community. The panel recommended that the Branch revise its mission statement to clarify the relevance of the mission to overall public health, wellness, and quality-of-life across the lifespan, and to better publicize its mission to congress, scientists, and the public. In so doing, the Branch could attract new investigators to the field, both as trainees and established scientists interested in interdisciplinary (and multidisciplinary) collaborations. Panel members envisioned that these partnerships would encourage scientific imagination and build new skill sets.

While noting infertility as a major public health issue, the panel members urged the Branch to focus on public health issues beyond infertility. For example, they noted that many prevalent conditions, such as obesity, diabetes, sexually transmitted diseases, and steroid abuse, and quality-of-life issues for women, such as pelvic floor disease and aging, were intimately tied to reproductive health. The Branch mission recognized that reproductive health influenced overall wellness, but should further emphasize that reproductive health affects not only the individual, but also the long-term wellness of their families, potentially for generations to come. RSB staff explained that studies on PCOS, particularly in adolescents, spoke directly to the link between ovarian function (specifically hyperandrogenism), obesity, and insulin regulation, and that the international consortium to study egg quality addressed the potential adverse effects of ART, as well as the trans-generational effects of reproductive health in the broadest sense. Staff added that results from research on spermatogonial stem cells had potentially broad implications for understanding of the molecular mechanisms of adult stem cell biology, for developing strategies to preserve fertility after pediatric cancer treatment, and for developing alternative adult stem cell populations for stem cell therapies.

The panel identified a number of areas with great potential for advancing reproductive science over the next four years. These areas included:

- Continued focus on reproductive genetics, particularly with regard to identification of disease genes (see the *Other Priority Areas* portion of this section of the report)
- Stem cell biology
- Antecedents of adult disease, including the role of gametes and prepubertal health status
- Reproductive health's impact on the quality-of-life throughout the lifespan of men and women, including the promotion of outcomes-based research efforts and epidemiological research

The panel also encouraged the RSB to continue supporting the WRHR Career Development Program and the recently launched MRHR Career Development Program to foster translation of basic science advances into clinical practice in female and male reproductive health. In addition, the panel recommended that the Branch continue to promote greater interaction between the RMN and the SCCPRR, noting that observational studies on the use of metformin in women with PCOS, a study supported by the SCCPRR, formed the basis for the pregnancy and PCOS randomized clinical trial conducted by the RMN.

The panel strongly encouraged the Branch to focus on complex diseases, including polygenic disorders and those with contributions from both genetic and environmental components. RSB staff noted that the Branch currently funded studies of the genetics of twinning, endometriosis, cryptorchidism, POF, PCOS, and reproductive aging. The panel also encouraged more epidemiological and outcomes-based studies, but realized the logistical obstacles that such studies posed. In particular, the panel suggested outcomes-based research as a vehicle to promote greater interactions with other NICHD Branches.

The panel urged the Branch to continue maintaining the balance between investigator-initiated research and center programs, particularly in times of zero-growth budgets. Members explained that the Branch functioned very efficiently and cost effectively, especially given the vast array of programs and mechanisms that it supports. They praised the Branch for establishing the SCCPRR as an exemplary program of translational research anchored by extensive interactions between basic and clinical scientists. In addition to conducting research, they acknowledged that the SCCPRR supported a variety of center-wide and world-wide resources for the scientific community and recommended that staff consider establishing other regional/national core facilities through the Program. The panel also praised the RSB for its active role in evaluating its center programs and encouraged staff to continue monitoring the effectiveness of these programs, which play a vital role in carrying out the Branch's and the Institute's mission.

Lastly, the panel suggested increasing collaboration with other NICHD Branches, other NIH Institutes, Centers, and Offices, and the private sector. Such collaborations were essential not only from a fiscal standpoint, but also from a scientific perspective given the mission overlap of many emerging scientific areas. In this regard, RSB staff described a few of its most recent collaborations:

- The Branch is collaborating with other Branches within the Center for Population Research on a non-hormonal male contraceptive initiative.
- The Branch recently co-sponsored a workshop with the Pregnancy and Perinatology Branch on adverse outcomes of ART.
- Over the years, the RSB has co-sponsored a number of workshops, conferences, and funding initiatives with the ORWH and the NCMHD in areas vital to women's and minority health.
- The RSB has maintained a presence on the NIH Stem Cell Task Force and has co-sponsored several trans-NIH initiatives on training in the use of hESC lines.
- Wyeth has agreed to make its data on epididymal gene expression available to all investigators through the Mammalian Reproductive Genetics Database.

FUTURE DIRECTIONS AND CHALLENGES

Technological breakthroughs and the fast pace of scientific advances make this an exciting time for reproductive science. The challenge for the next four years, therefore, is to provide opportunities to enable the community of scientists to continue unraveling the great "miracle" of reproduction, and to take advantage of the technological breakthroughs to answer previously unanswerable questions. Given these times of limited budget growth, the Branch will have to prioritize its agenda. In addition to identifying priority research, technology, and investigator recruitment and retention issues, comments from the expert panel and from within the Branch

suggest that the RSB needs to "repackage" itself to adequately convey the significance of its research advances, moving beyond fertility and infertility to the overall health and well-being of the public that it serves. Indeed, while assisted reproduction has enabled couples to have families, thereby enhancing quality-of-life, the physical pain, mental anguish, and psychosocial dysfunction that are sequelae of many reproductive diseases and disorders cannot be corrected by ART. Although clearly no small challenge, the Branch has already begun to address these issues by creating two programs with potential public health significance: Fertility Preservation, and Preconception Care. Both of these areas intersect various portfolios, thereby promoting a team oversight paradigm. The next two sections describe the rationale for these Programs.

Fertility Preservation Program

In the fall of 2005, the RSB announced the development of a new Fertility Preservation Program to:

- Develop a cadre of basic, clinical, and translational investigators to advance knowledge of the pathophysiology related to chronic disease and/or its treatment and disruption in reproductive function;
- Characterize individuals (adults and children) who are most likely to experience premature reproductive failure;
- Develop technology to more accurately measure ovarian damage or reserve, and relate those measures to reproductive failure, and
- Develop strategies to prevent the loss of fertility.

To accomplish its goals, the Fertility Preservation Program will encourage investigator-initiated applications and will develop a funding opportunity announcement, targeted for fiscal year 2009, that will be fostered through the inter-agency Fertility Preservation Working Group, which was convened by the Branch in the fall of 2005. The Working Group will host an advisory panel roundtable in early 2007 to seek recommendations on future research directions to help guide the fiscal year 2009 initiative. Until then, the RSB will continue to encourage research efforts within individual portfolios that can directly impact the success of this Program, such as the development of more effective cryopreservation and culturing methods for oocytes and ovarian follicles. Dr. Charisee Lamar will oversee the Fertility Preservation Program.

Preconception Care Program

New evidence indicates that the health and well-being of both women and men prior to a first conception or between pregnancies can have a profound impact on the health status of offspring for multiple generations. Accordingly, the RSB has embarked on a Preconception Care Program, which considers the importance of the pre- and peri-conception period on the health of offspring. Building on the hypothesis that adult disease prevalence can reflect problems encountered by the fetus (a.k.a., fetal origins of adult disease), the Branch has begun to identify adverse experiences of females prior to or shortly after conception that result in poor egg quality and subsequent deficits in the offspring through its Female Health and Egg Quality Consortium. These adverse events include stress and toxic exposures, as well as behaviors related to nutrition, exercise, and drug use. The Branch will also encourage and actively solicit applications that focus on paternal contributions to embryonic development, and on the harm male gametes suffer from environmental insults. As a corollary to these approaches, the Branch will seek to identify

biomarkers that relate the quality of gametes and preimplantation embryos to adverse long-term outcomes in fetuses and offspring. It is anticipated that findings involving non-primate and non-human primate animal models could be immediately transferred to humans. Drs. Estella Parrott and Richard Tasca will oversee the Preconception Care Program.

Other Priority Areas

In addition to the two overarching Programs mentioned earlier, the RSB, with input from the expert panel, identified additional priority areas related to research, technology/bioinformatics, and investigator recruitment and retention relevant to the Branch's activities for the next four years. These include (in no particular order):

- **Expand technological resources** for scientists in the field. DNA- and protein-microarray technology (to assess global transcriptional patterns and protein expression) and their applications to the study of reproduction and reproductive diseases and disorders are a high priority. Further development of reproductive genomic databases and tissue banks begun in the SCCPRR are also essential components of the Branch's future plans.
- **Increase efforts to encourage new investigators to pursue a career in the reproductive sciences**. To this end, the RSB will continue to stimulate recruitment and retention of new scientists, especially underrepresented minorities, into the scientific community through individual and institutional training and career development opportunities, and through research center programs. Branch staff will emphasize exposing reproductive scientists who have doctorate degrees to clinical research to facilitate translational research; staff will also seek to attract scientists from other disciplines to work with reproductive scientists on problems requiring multidisciplinary approaches. The Branch will also make efforts to attract clinician-scientists to careers in men's reproductive health through the newly established MRHR Career Development Program.
- **Provide a means to retain physician-scientists in research careers.** The Branch will seek to develop a bridging grant program, with relevant societies and foundations that will provide interim support for clinician-scientists seeking renewal of their initial R01 support.
- **Continue to expand and emphasize research addressing the etiology of women's reproductive diseases**, such as uterine leiomyoma, which continues to be poorly understood and exhibits racial and ethnic disparities in the incidence of disease. The Branch will also increase efforts to stimulate research on sexual dysfunction in women and on the role of adipose tissue and its products (e.g., adipokines) in reproductive function and dysfunction.
- **Focus greater attention on diagnosing reproductive and gynecologic diseases of pre- and post-pubertal adolescents**. This area is critical in terms of overall health and quality-of-life because many reproductive diseases and disorders (such as PCOS and endometriosis) are not identified until the completion of sexual maturation (pubarche). If these disorders could be identified early on, interventions could be developed to reduce or ameliorate the incidence of disease in adulthood.
- **Promote the concept of male reproductive health** as encompassing not only causes of and treatments for male infertility and advances in male contraception, but also male sexual dysfunction and the use/abuse of steroid hormones for the age-related decline in male fertility. Likewise, there is a need to increase research into the normal role of the sex accessory glands in fertility, as well as in non-reproductive functions. Immunological aspects of the male reproductive tract, particularly as related to the role of the epididymis as a

protein reservoir that protects the male reproductive tract from infection, are also research priorities.

- **Support studies that move beyond the correlation of a causative gene with a reproductive disease or disorder to examine complex polygenic disorders, gene-environment interactions, epigenetic modifications, and potential intergenerational effects on reproductive health and fertility.** Approaches could include: large population-based studies to elucidate the genetic components of complex disorders and traits such as twinning, PCOS, POF, and endometriosis; and epidemiological studies to assess the outcomes of ART, particularly potential epigenetic effects and the reproductive sequelae in both the mother and child. The Branch will also place a greater emphasis on male reproductive genetics, particularly on the reproductive and intergenerational effects of Y-chromosome deletions and polymorphisms, Klinefelter syndrome, and cryptorchidism.
- **Encourage research on all aspects of stem cell biology**, both embryonic and adult, to shed light on normal gamete development and development of other reproductive tissues. The Branch will also use animal models to improve the means to diagnose genetic diseases in preimplantation ESCs.
- **Pursue investigations at the intersection of the immune and endocrine systems** because these systems share many common molecules and intracellular signaling pathways. Greater emphasis in this area is likely to have payoffs in elucidating the origins of reproductive diseases and disorders that impact fertility.
- **Encourage research using systems and integrative biological approaches.** Systems biology, i.e., the coordinated study of a biological system, is necessary to gain a comprehensive understanding of regulatory networks that influence reproductive function. This type of research involves:
  - Identifying genes, proteins, and other small molecules comprising the regulatory network;
  - Perturbing each pathway component and assessing the global cellular response;
  - Integrating the observed responses with existing models of component interaction using appropriate computational models; and
  - Formulating new hypotheses to explain observations not predicted by the model.

FIGURES AND TABLES

FIGURE 1: SPECIALIZED COOPERATIVE CENTERS PROGRAM IN REPRODUCTION RESEARCH (SCCPRR) SITES

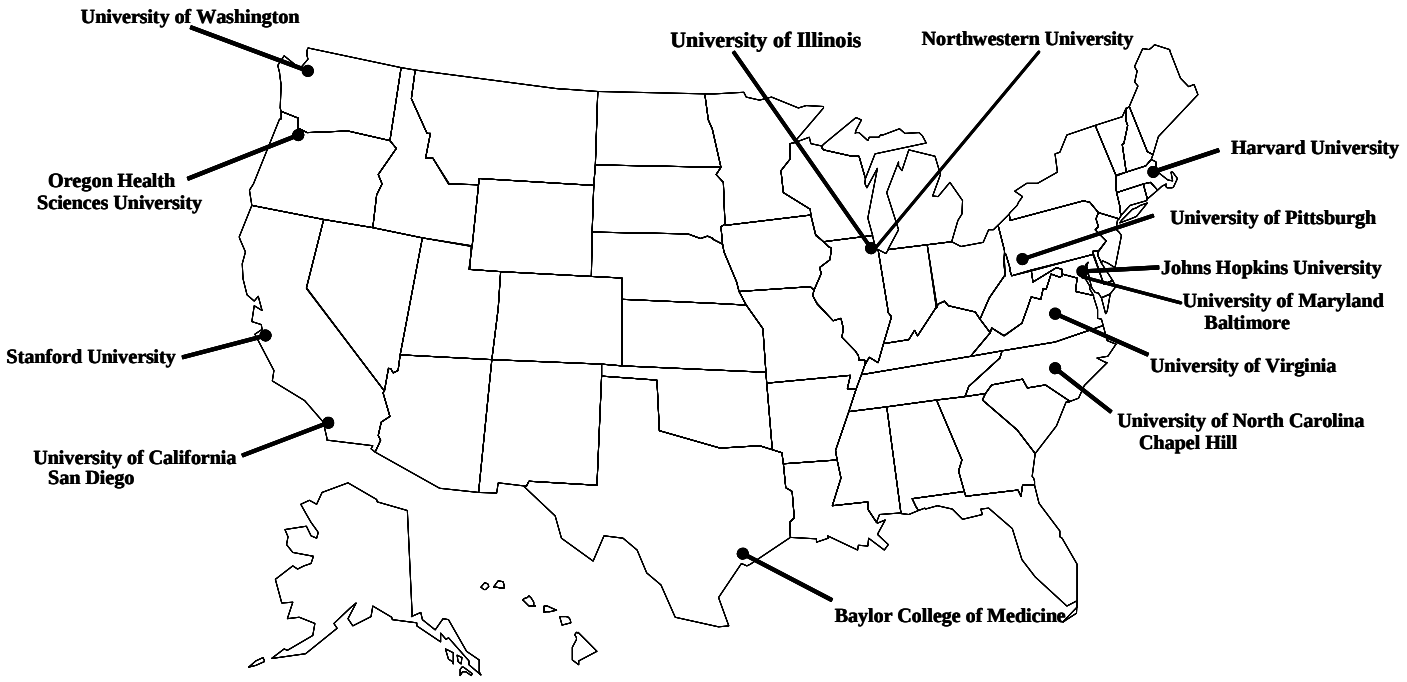

FIGURE 2: NATIONAL COOPERATIVE REPRODUCTIVE MEDICINE NETWORK (RMN) SITES

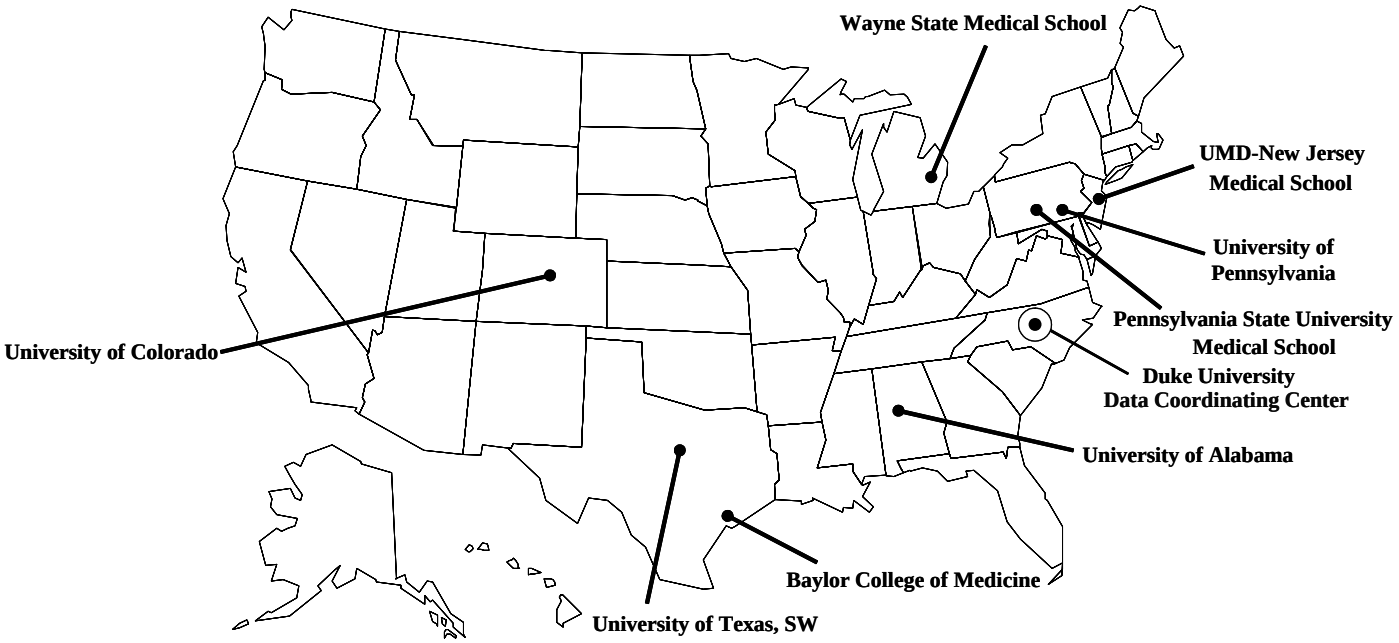

FIGURE 3: WOMEN'S REPRODUCTIVE HEALTH RESEARCH (WRHR) CAREER DEVELOPMENT PROGRAM SITES

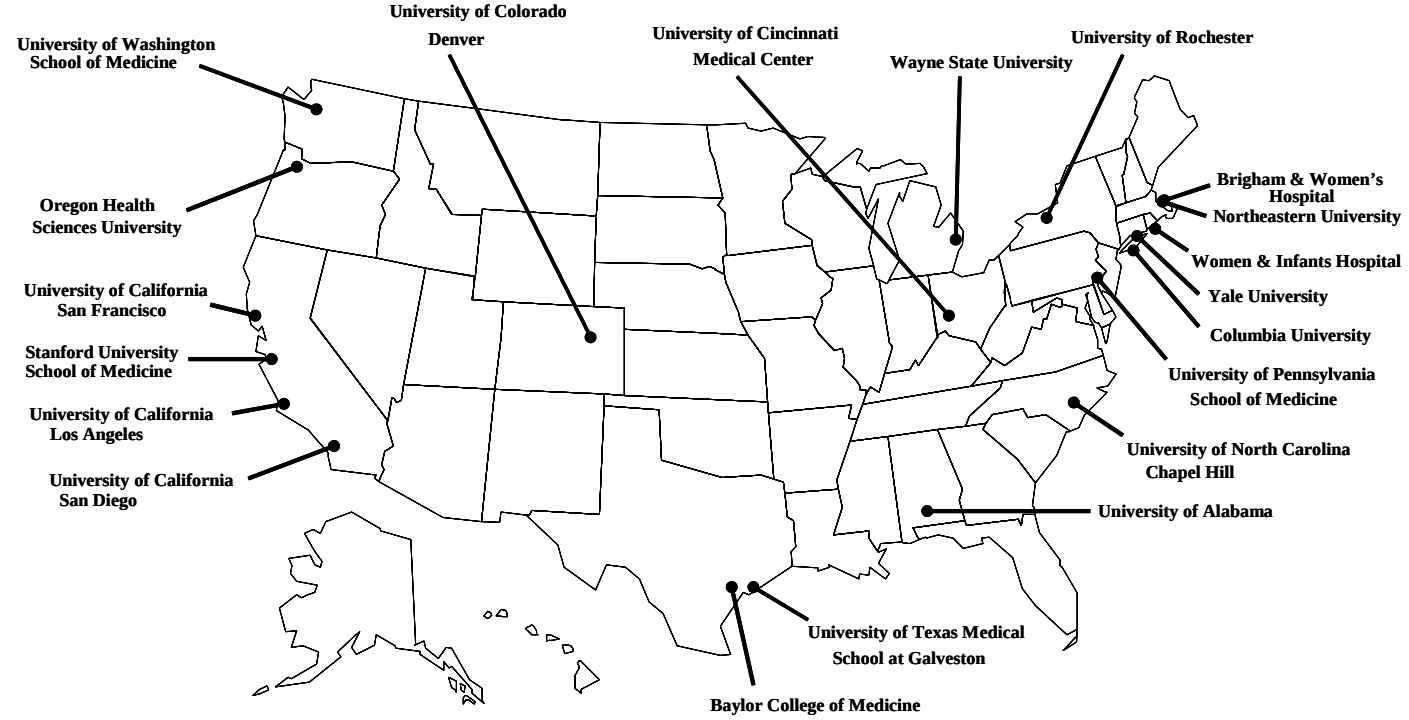

FIGURE 4: RSB-FUNDED INSTITUTIONAL TRAINING AWARDS (T32)

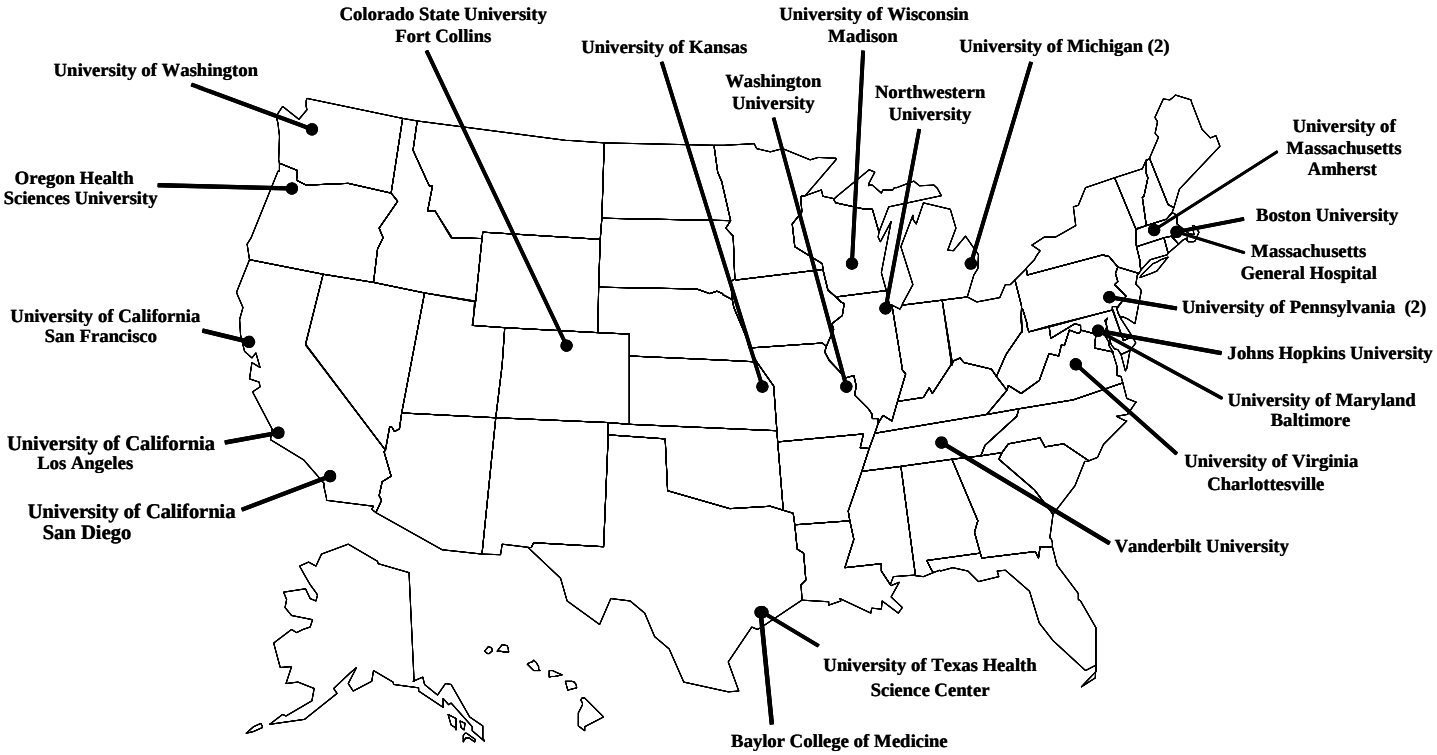

FIGURE 5: RSB FUNDS IN CURRENT AND CONSTANT DOLLARS, FISCAL YEAR 1996 THROUGH FISCAL YEAR 2006

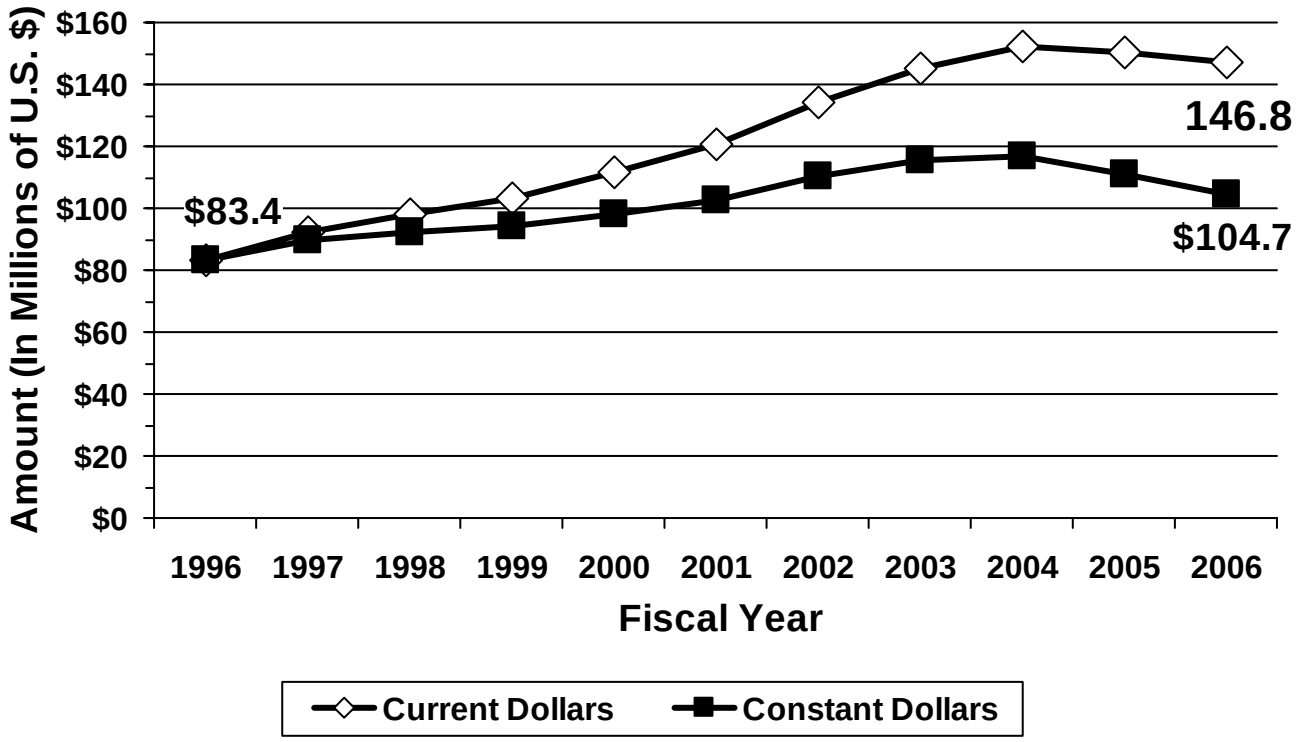

FIGURE 6: RSB FUNDING BY SUPPORT MECHANISM, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

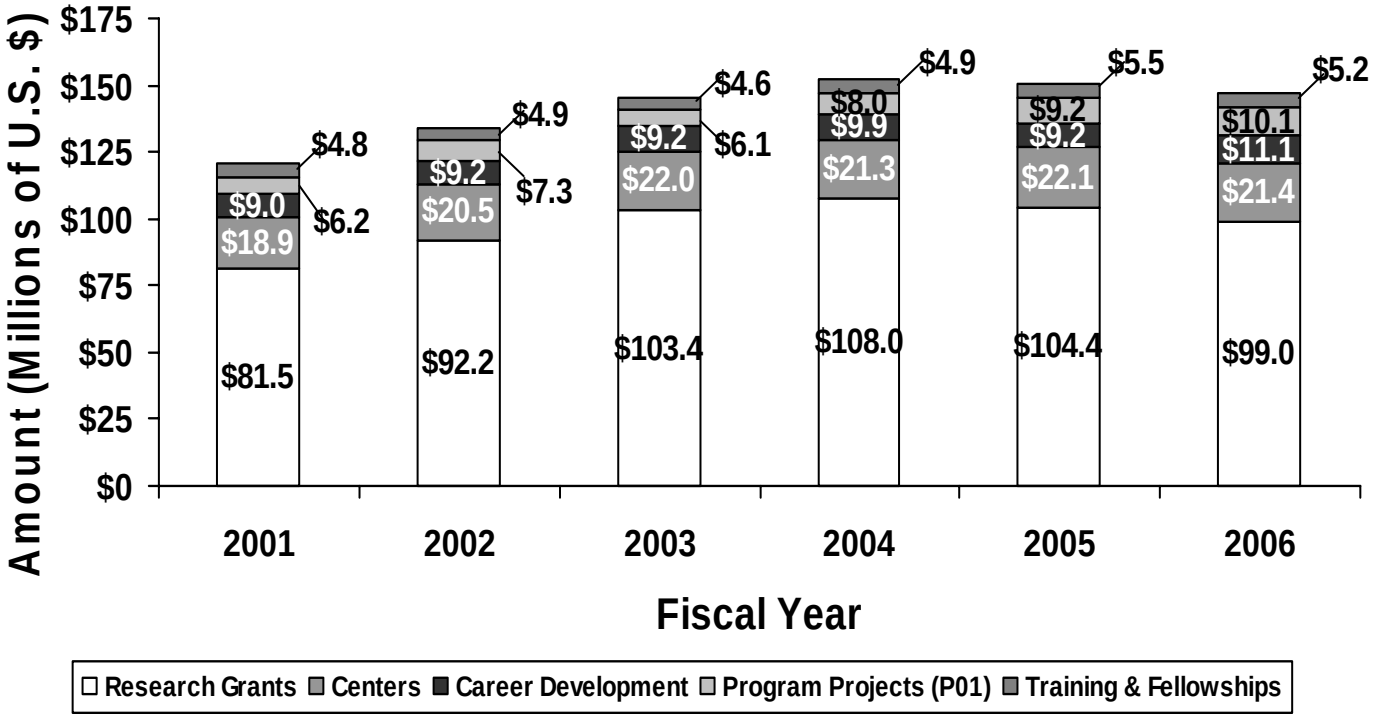

FIGURE 7: NUMBER OF BRANCH PROJECTS BY SUPPORT MECHANISM, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

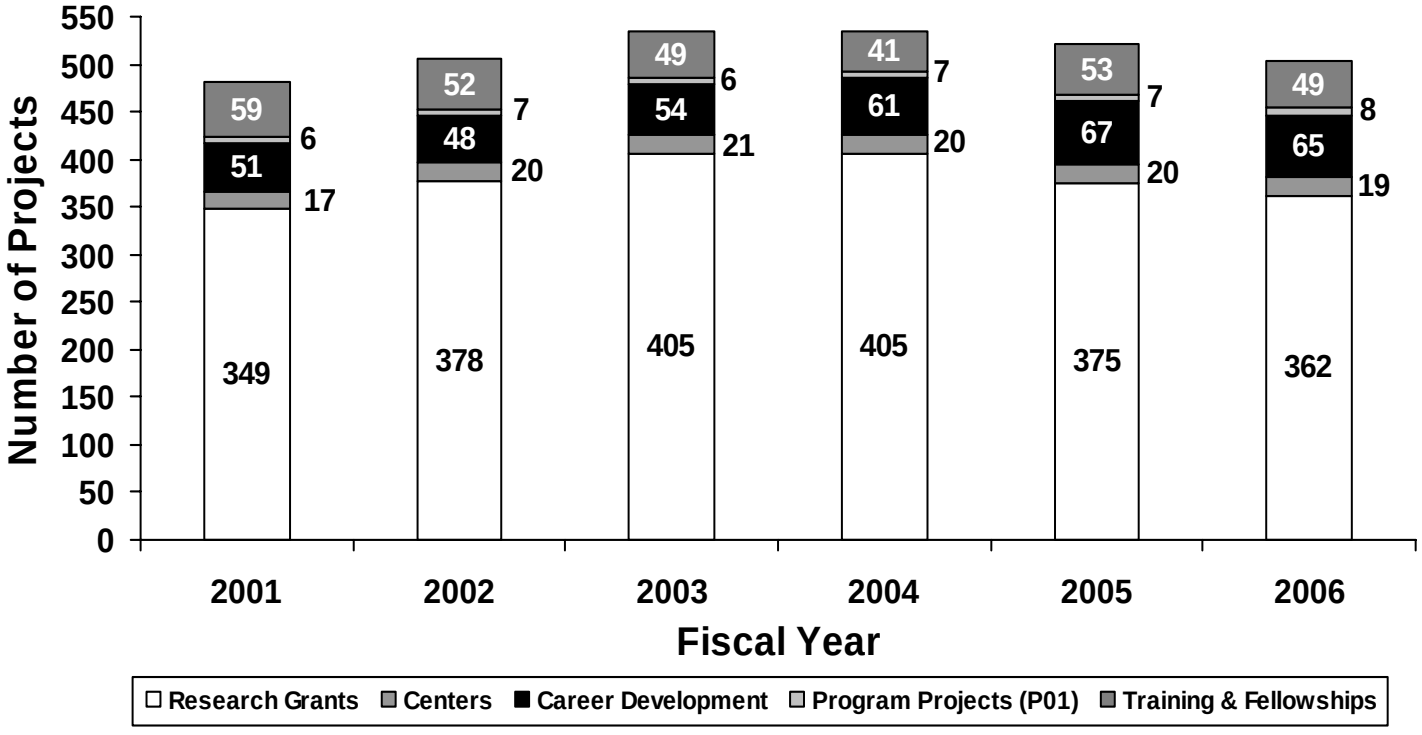

FIGURE 8: RSB FUNDING BY SUPPORT MECHANISM, FISCAL YEAR 2006

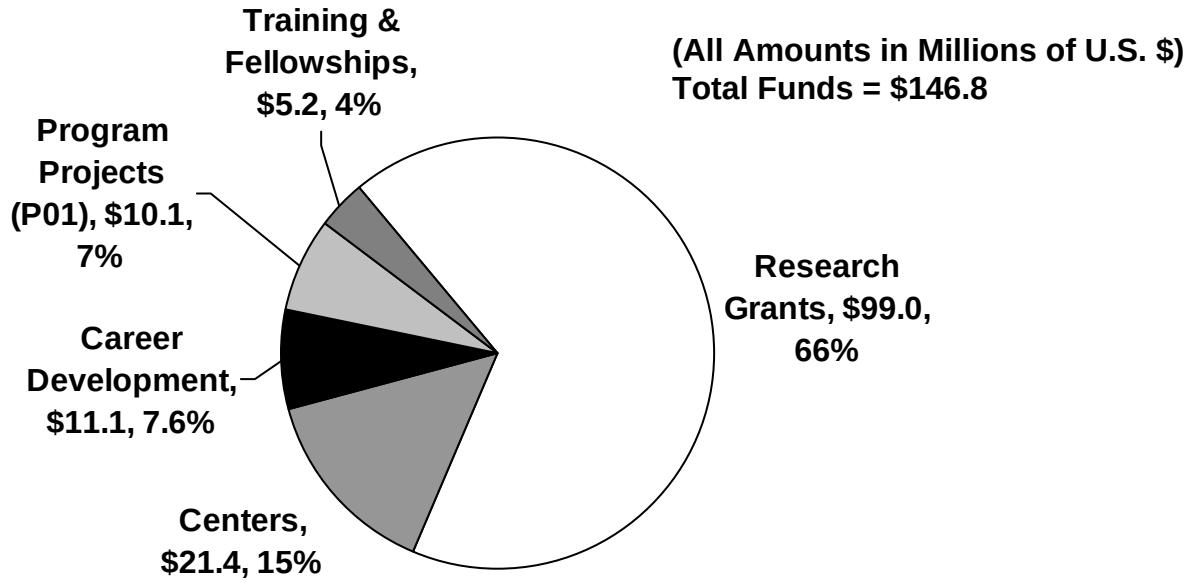

FIGURE 9: NUMBER OF BRANCH PROJECTS BY SUPPORT MECHANISM, FISCAL YEAR 2006

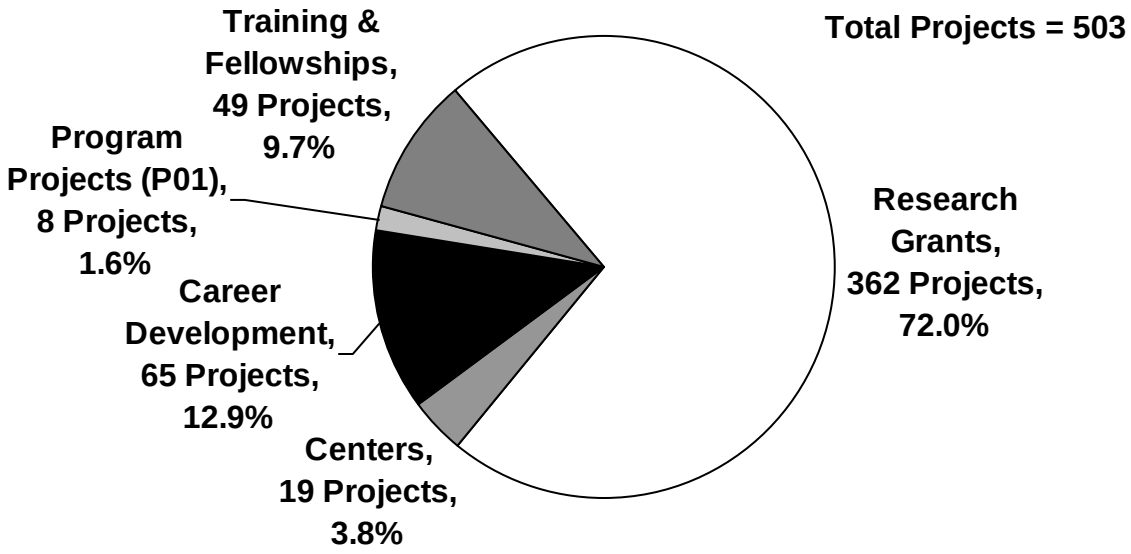

FIGURE 10: RSB FUNDING BY RESEARCH PROGRAM, FISCAL YEAR 2004 THROUGH FISCAL YEAR 2006

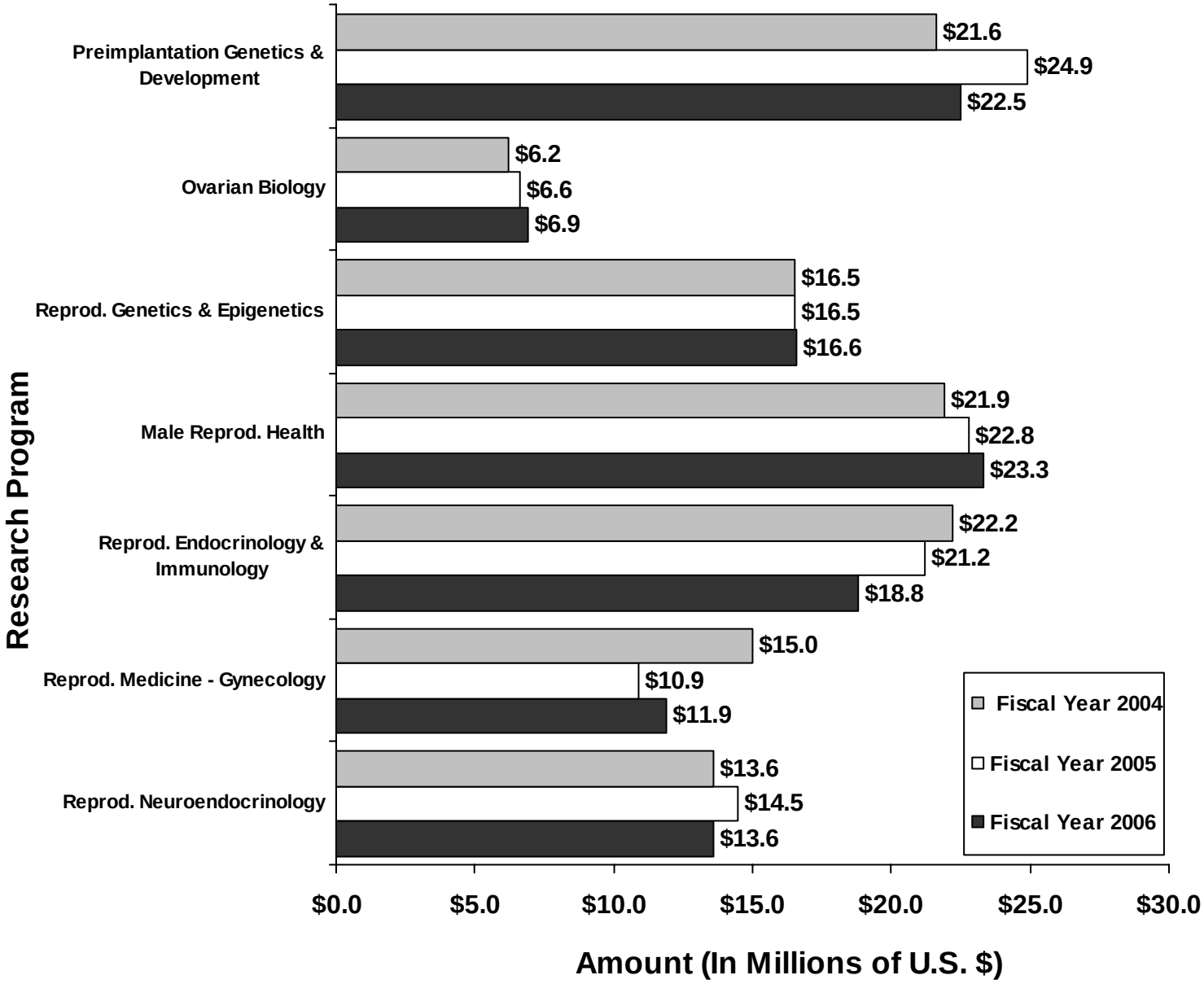

FIGURE 11: NUMBER OF BRANCH PROJECTS BY RESEARCH PROGRAM, FISCAL YEAR 2004 THROUGH FISCAL YEAR 2006

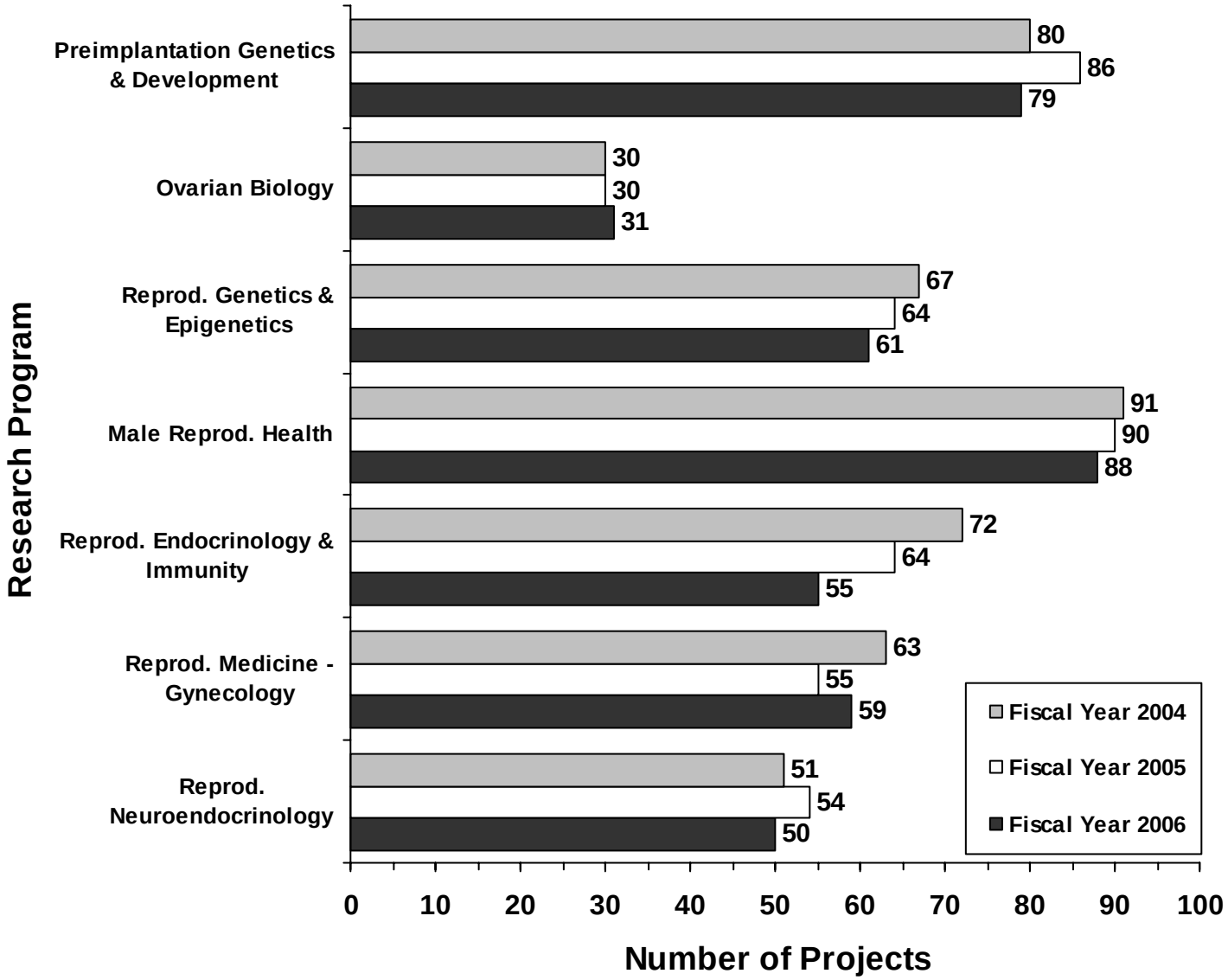

FIGURE 12: NUMBER OF RESEARCH PROJECT GRANTS BY PROGRAM, FISCAL YEAR 2004 THROUGH FISCAL YEAR 2006

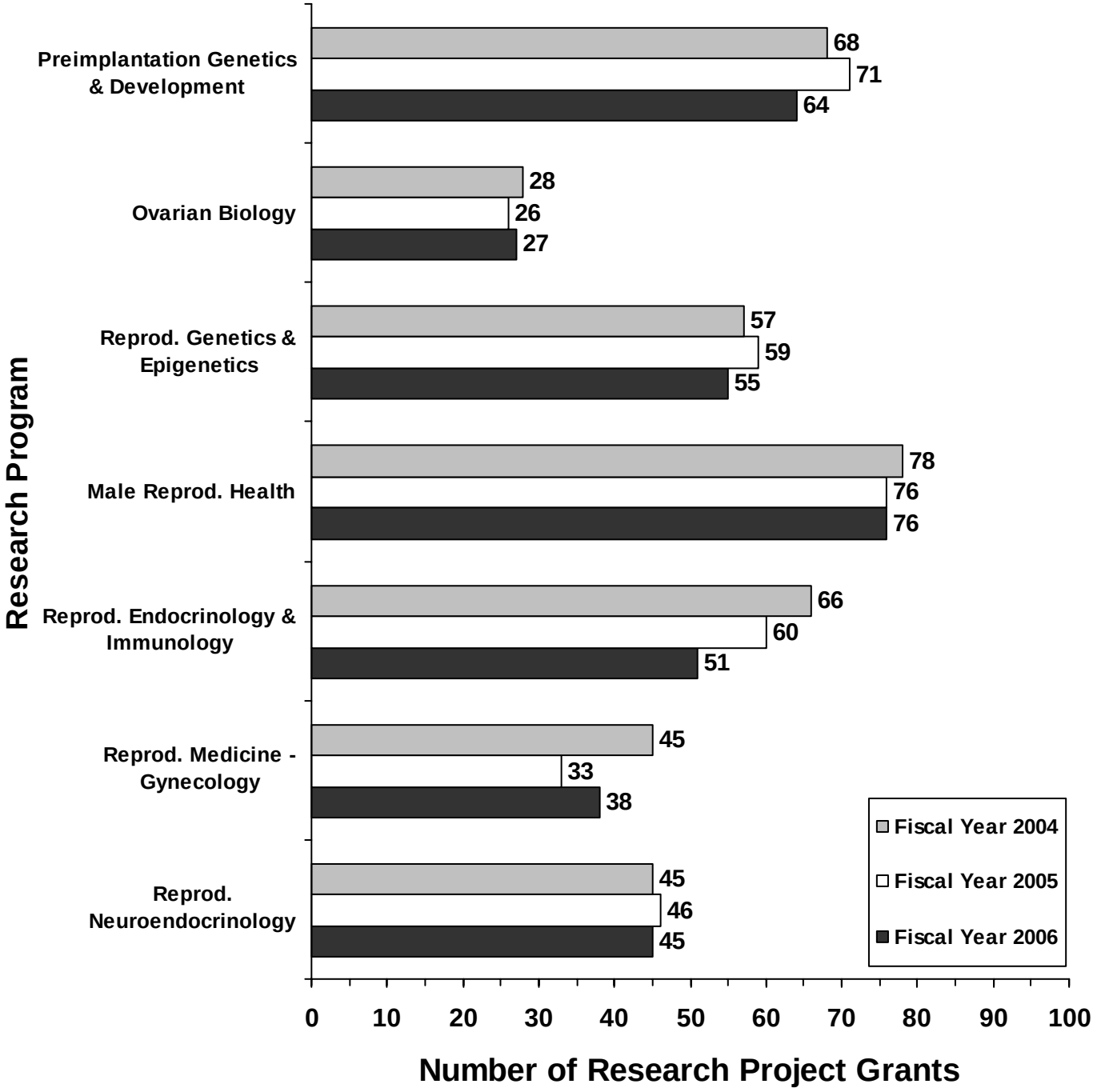

TABLE 1: RSB PORTFOLIO RESEARCH AREA SUBCATEGORIES

Program Officer Initials	Subcategory	Code
CL	Building Interdisciplinary Research Careers in Women's Health	IWHR
CL	Fertility Preservation in Cancer Survivors	FPCS
CL	Metabolic Fuel Regulation of Reproduction	MFRR
CL	Gonadotropin Regulation	GTHS
CL	Mechanism of GnRH Action	GNAC
CL	Neural Control of Reproduction	NEUR
CL	Prolactin and Other Peptide Hormones	PROL
CL	Reproductive Behavior	BEHV
EP	Clinical Studies and Clinical Trials	CSCT
EP	Female Reproductive Tract: Development (Vulva, Vagina, Oviduct, Ovary, Uterus)—Clinical	DFRD
EP	Etiology, Prevention, and Treatment Aspects of Female Infertility	EPTI
EP	Female Reproductive Science Training Programs	RSTP
EP	Female Reproductive Tract: Diseases and Disorders (Vulva, Vagina, Oviduct, Ovary, Uterus)	FRDD
EP	Female Sexual Function and Dysfunction	FSFD
EP	Reproductive Medicine Network	RMN
KY	Female Reproductive Tract (Oviduct, Uterus, and Vagina)—Basic	TRAR
KY	Implantation of Blastocyst, Trophoblast-Uterine Interactions	IMPL
KY	Mechanism of Action of Gonadotropins, Peptide Hormones, Receptors	GONA
KY	Reproductive Immunology, Cytokines	IMMU
KY	Steroids: Synthesis, Secretion, Metabolism, Mechanism of Action	STER
RT	Cloning, Reprogramming	CLON
RT	Embryonic Germ/Stem Cell Research	STEM
RT	Embryos, Genetics, Epigenetics	EMBR
RT	Germline, Germ Cells	GERM
RT	Minority Institutions Reproductive Science Centers	MINC
RT	Oocytes, Genetics, Epigenetics, Quality	OOCY
RT	Preimplantation Genetic Diagnosis	PGDR
ST	Basic Ovarian Biology	BOVB
ST	Assisted Reproductive Technology	ARTE
ST	Epigenetics of Gametogenesis and X-Chromosome Inactivation	EPIX
ST	Genetics of Infertility and Reproductive Aging	GIFA
ST	Sex Determination and Differentiation	SXDD
ST	Training, T32s	TRNG
TR	Basic Testes Physiology	TEST
TR	Epididymis	EPID
TR	Fertilization	FERT
TR	Male Tract (other than Testes and Epididymis)	TRAC
TR	Sperm Function	SPFX
TR	Spermatogenesis	SPER

Program Officer Initials	Subcategory	Code
	Conference	CONF
	Career Development	KDEV
	Research Centers	CENT

FIGURE 13A: RSB FUNDING BY RESEARCH SUBCATEGORY, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

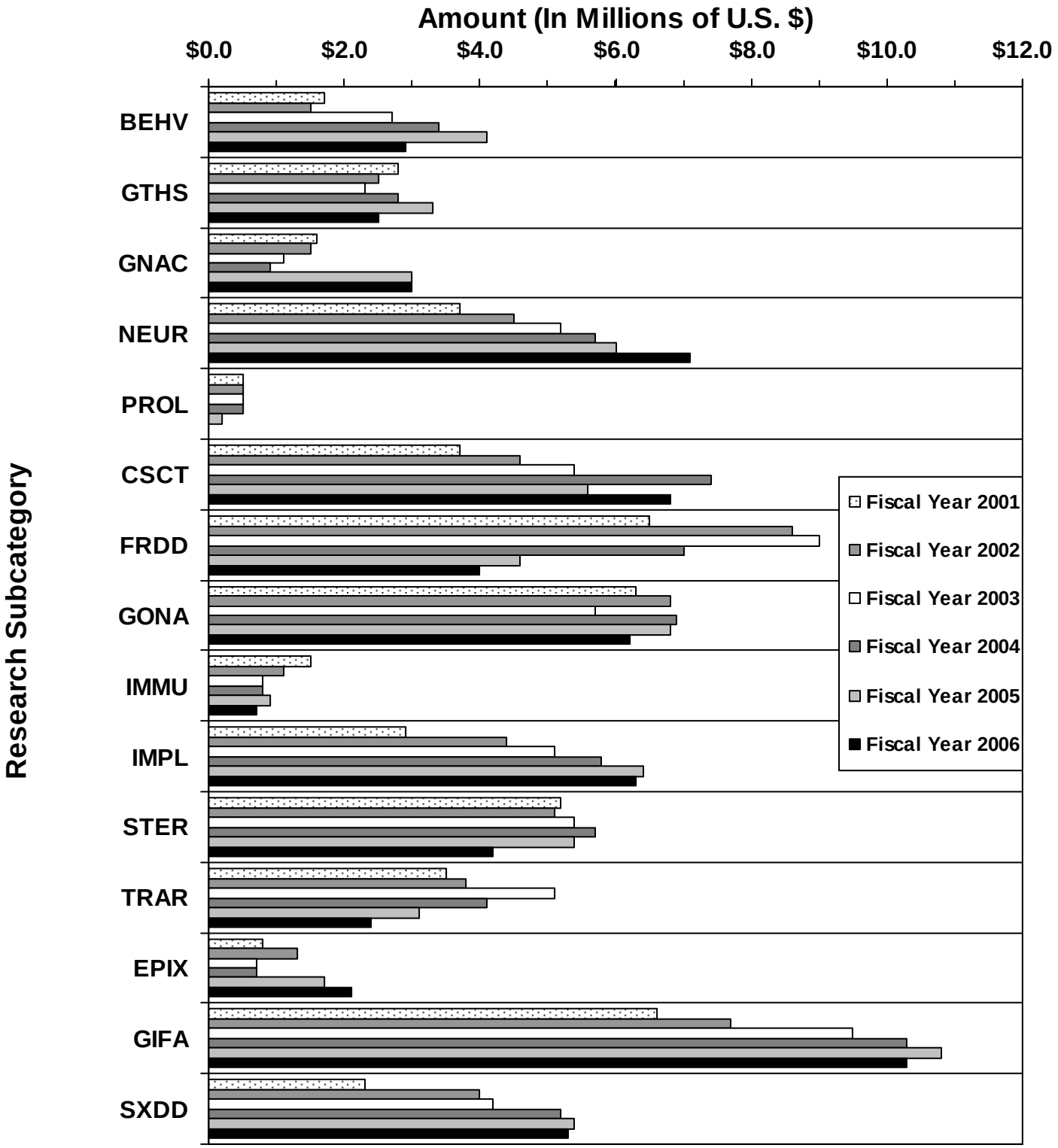

FIGURE 13B: RSB FUNDING BY RESEARCH SUBCATEGORY, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

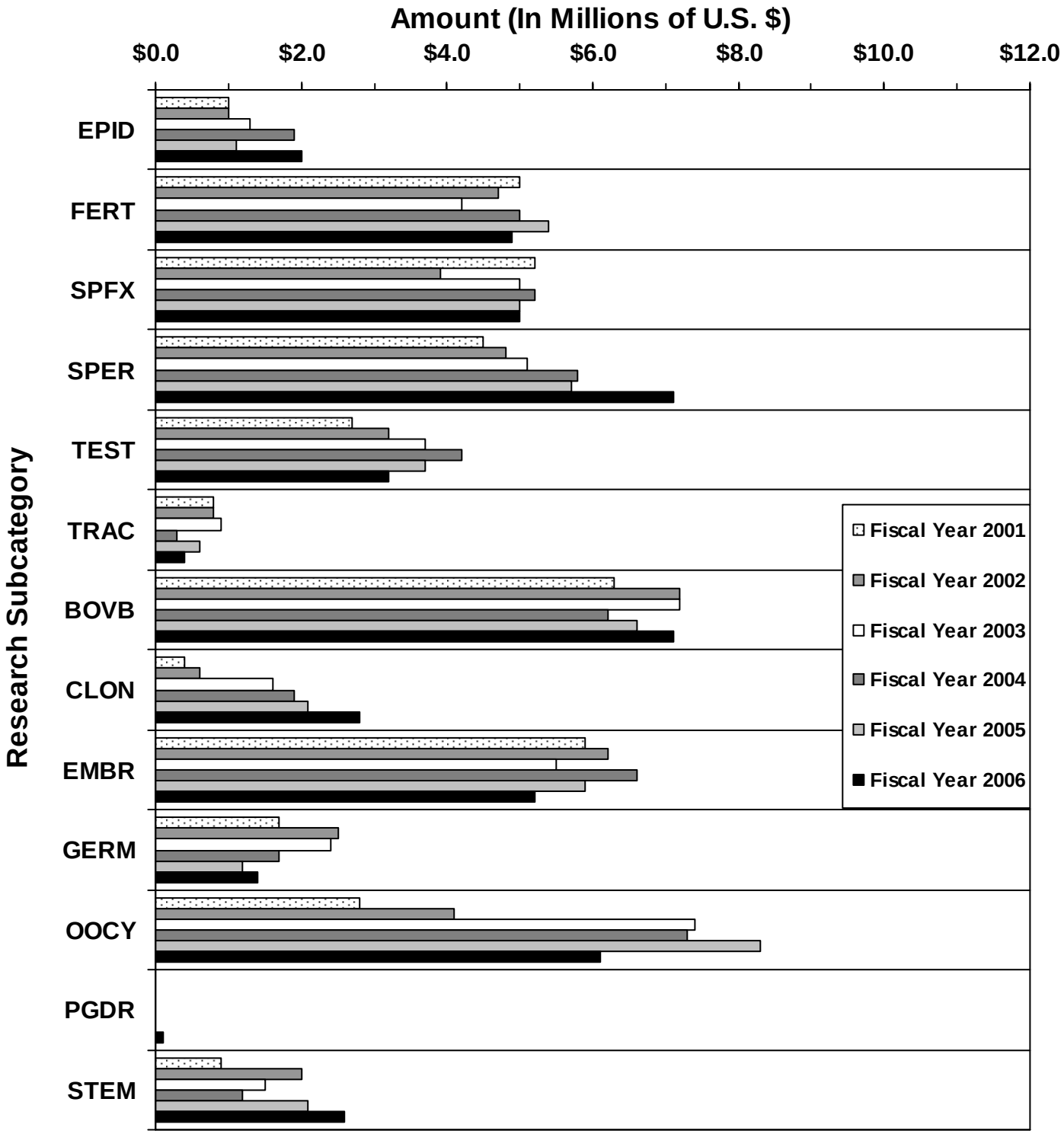

FIGURE 14A: NUMBER OF BRANCH PROJECTS BY RESEARCH SUBCATEGORY, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

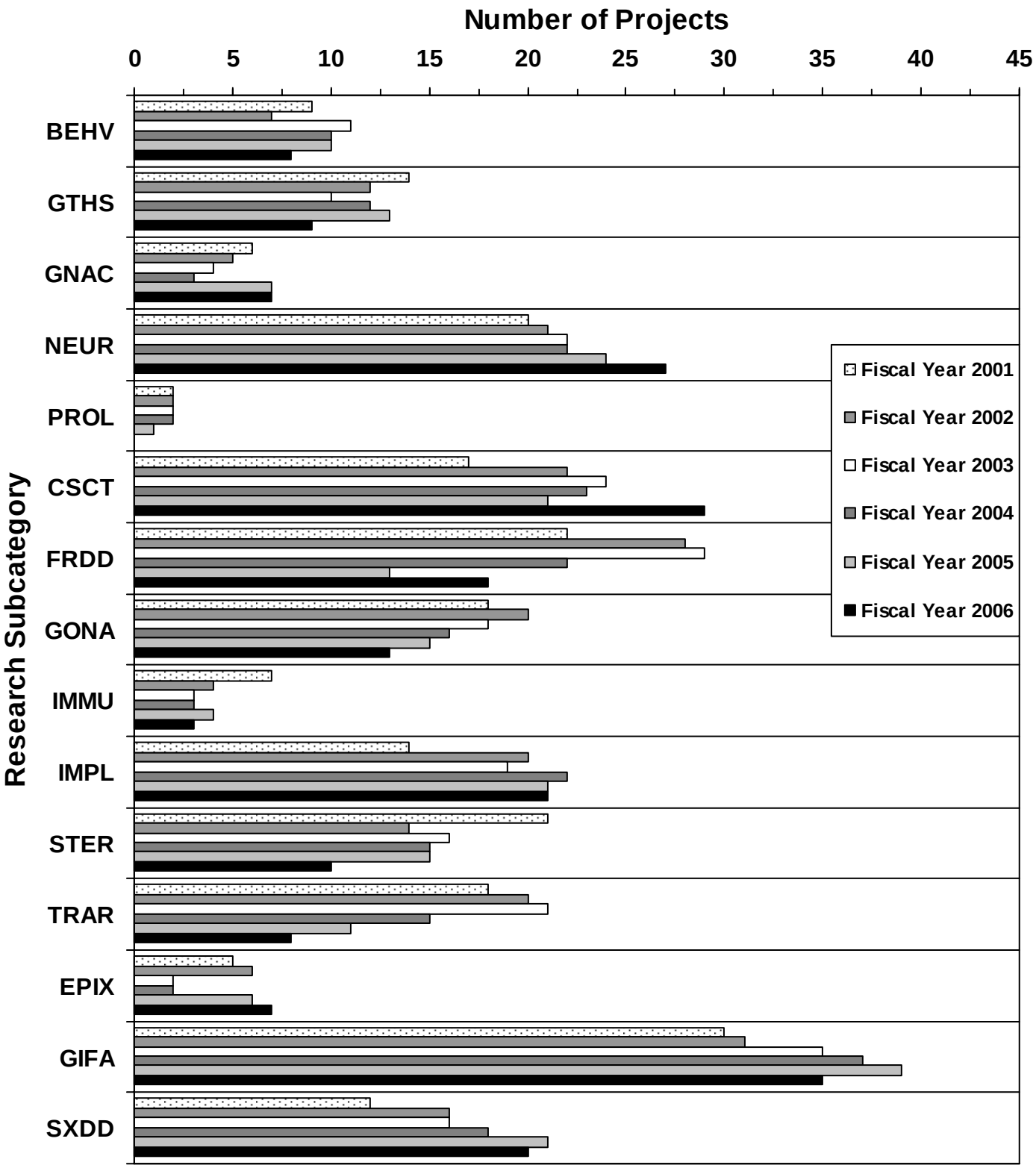

FIGURE 14B: NUMBER OF BRANCH PROJECTS BY RESEARCH SUBCATEGORY, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

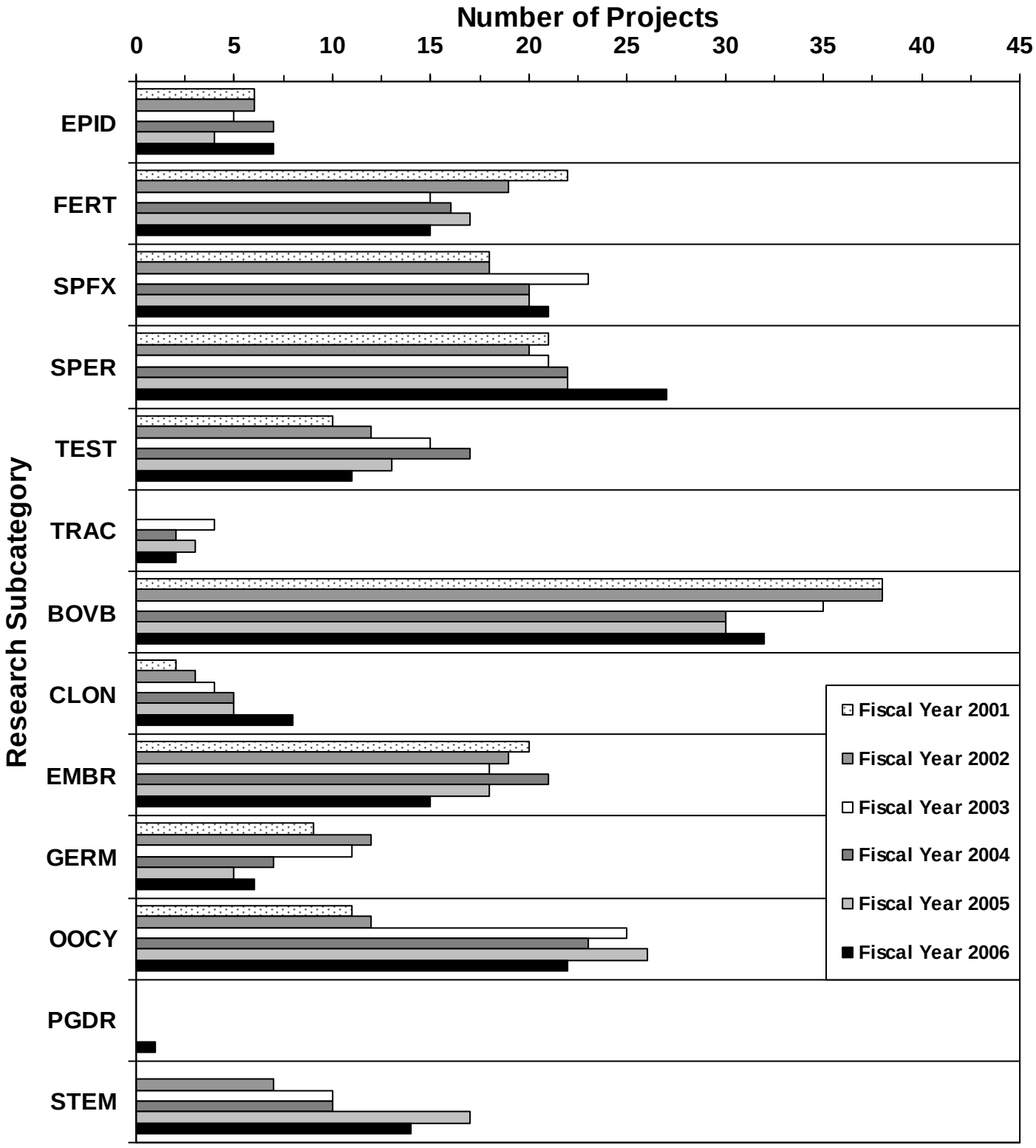

FIGURE 15A: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE NEUROENDOCRINOLOGY PROGRAM, BY FISCAL YEAR

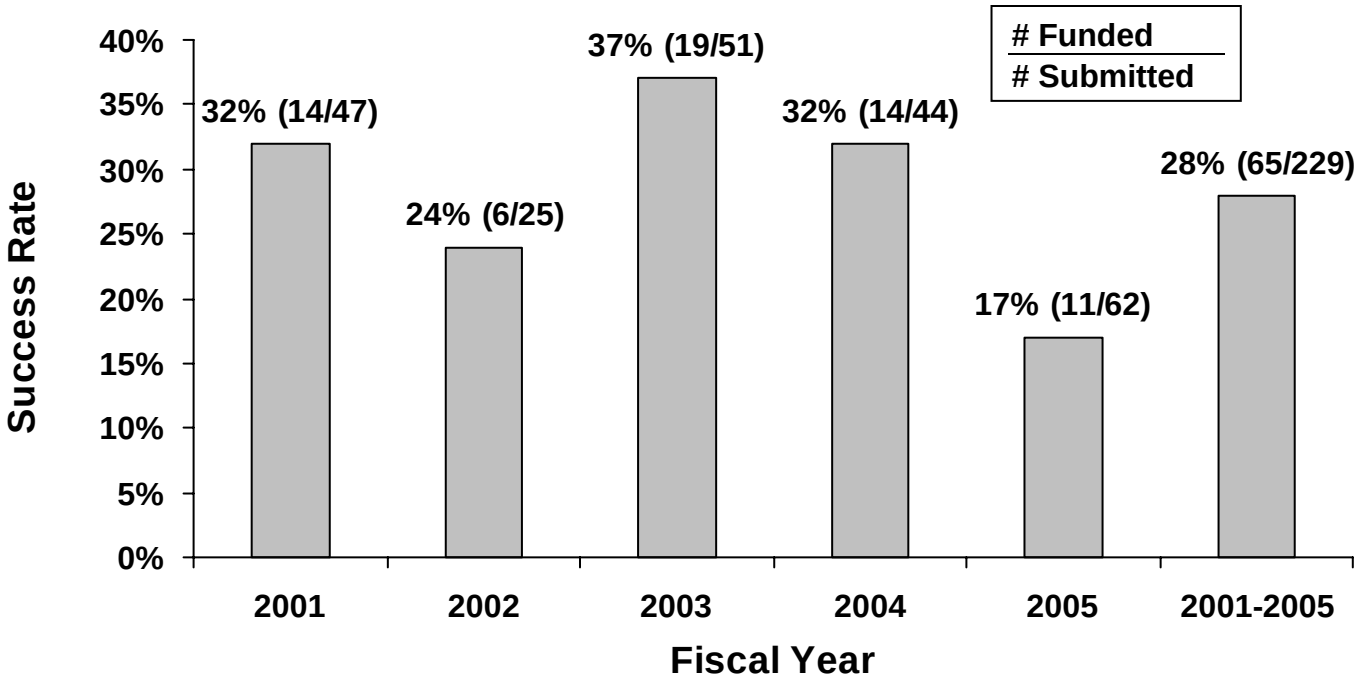

FIGURE 15B: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE NEUROENDOCRINOLOGY PROGRAM, BY SUBCATEGORY

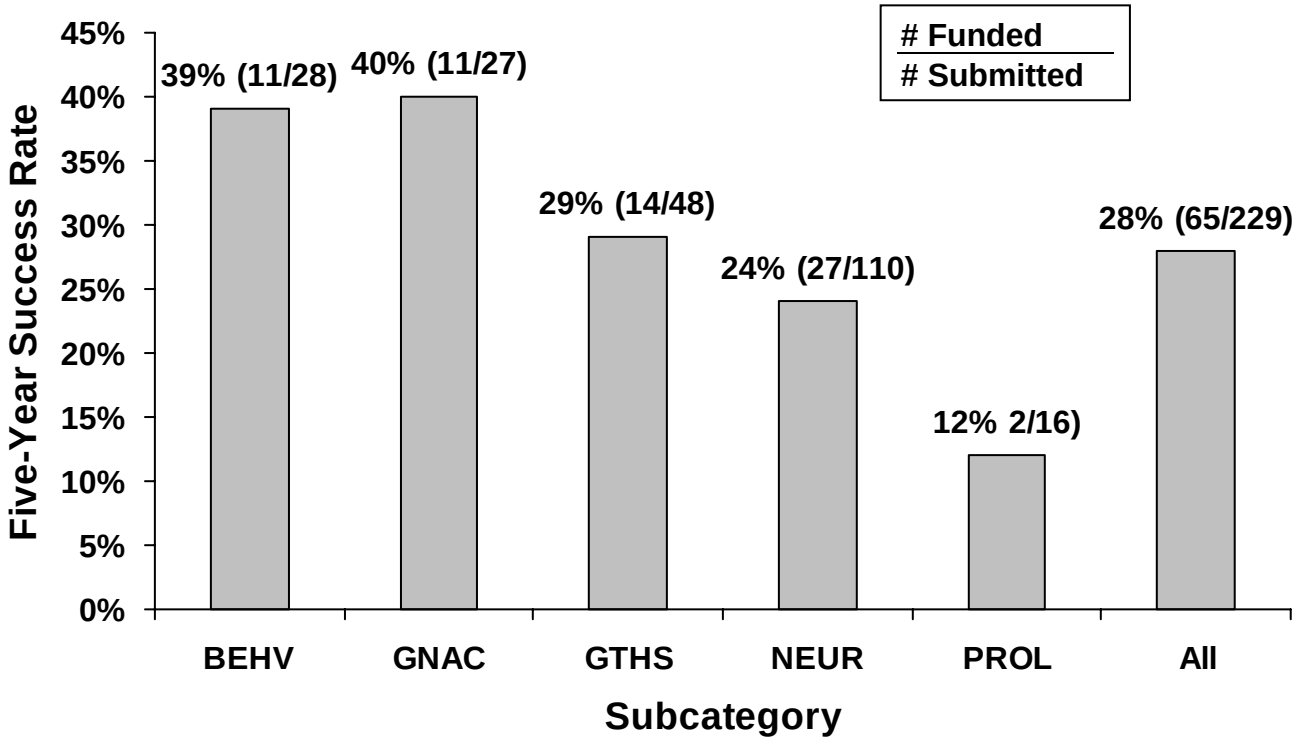

FIGURE 16A: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE MEDICINE-GYNECOLOGY PROGRAM, BY FISCAL YEAR

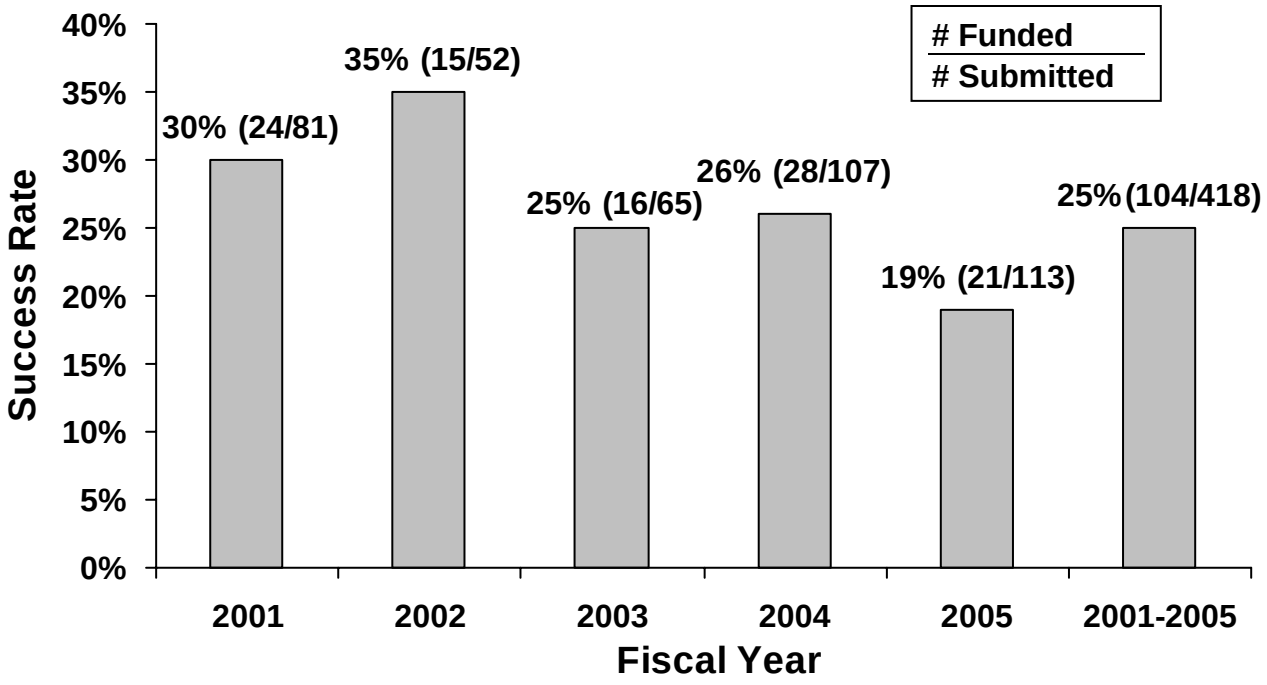

FIGURE 16B: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE MEDICINE-GYNECOLOGY PROGRAM, BY SUBCATEGORY

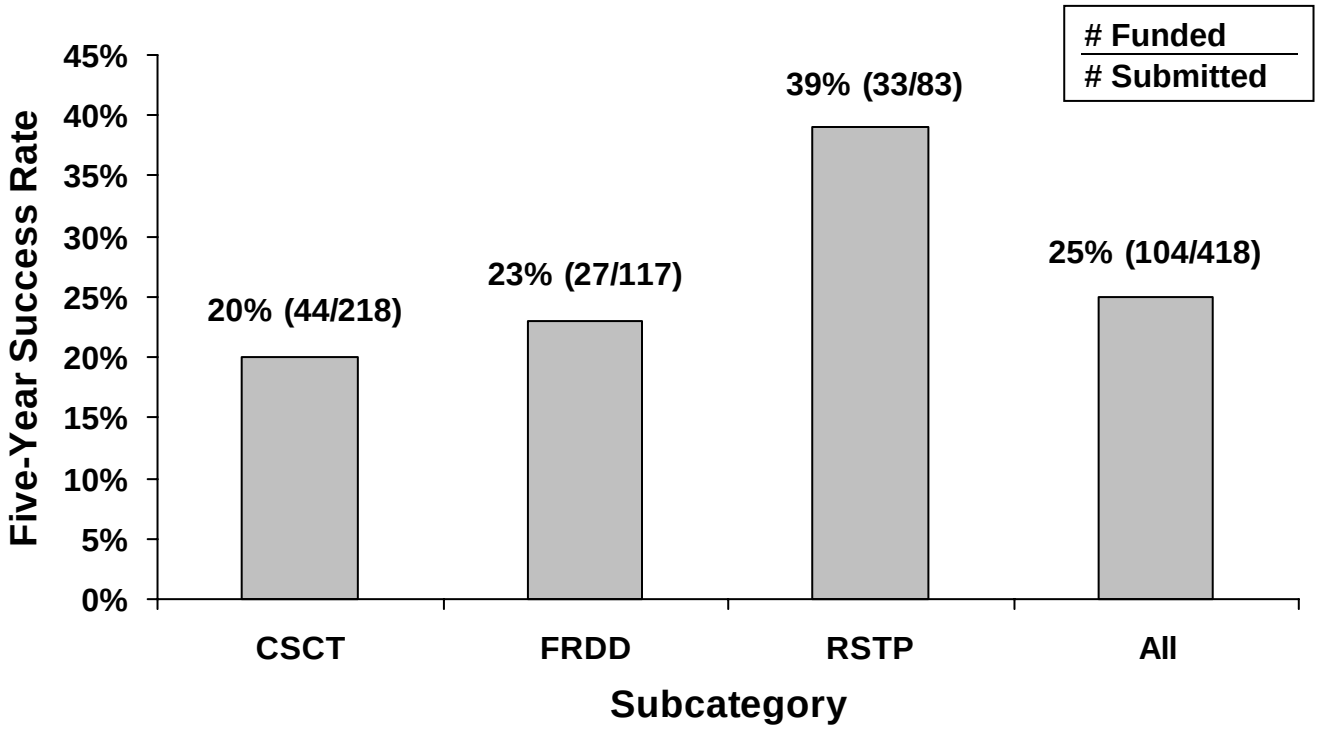

FIGURE 17A: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE ENDOCRINOLOGY AND IMMUNOLOGY PROGRAM, BY FISCAL YEAR

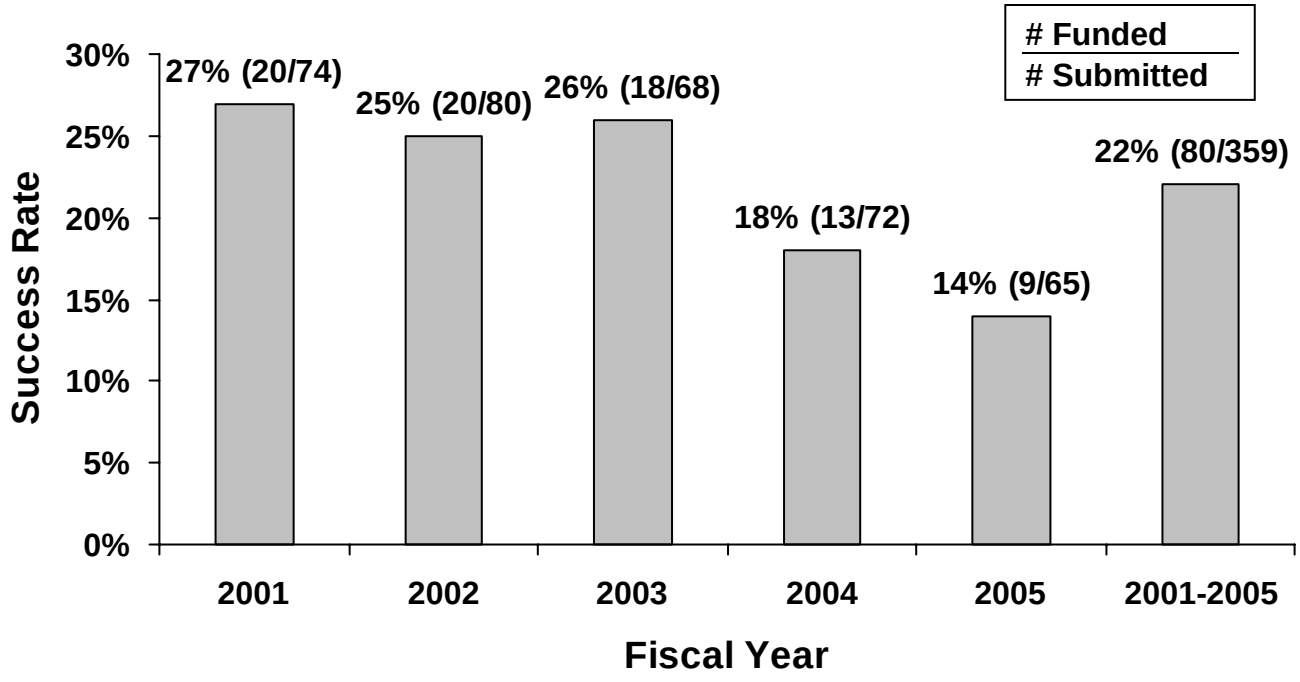

FIGURE 17B: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE ENDOCRINOLOGY AND IMMUNOLOGY PROGRAM, BY SUBCATEGORY

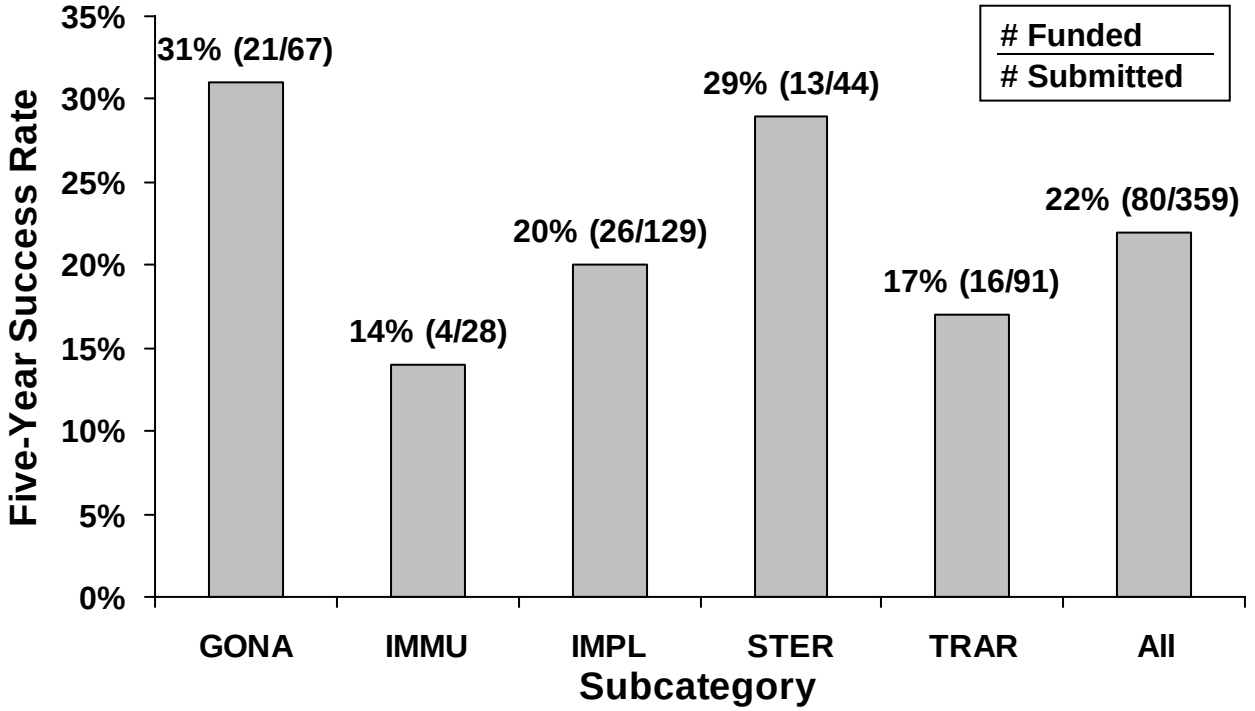

FIGURE 18A: SUCCESS RATE OF APPLICATIONS TO THE PREIMPLANTATION GENETICS AND DEVELOPMENT PROGRAM, BY FISCAL YEAR

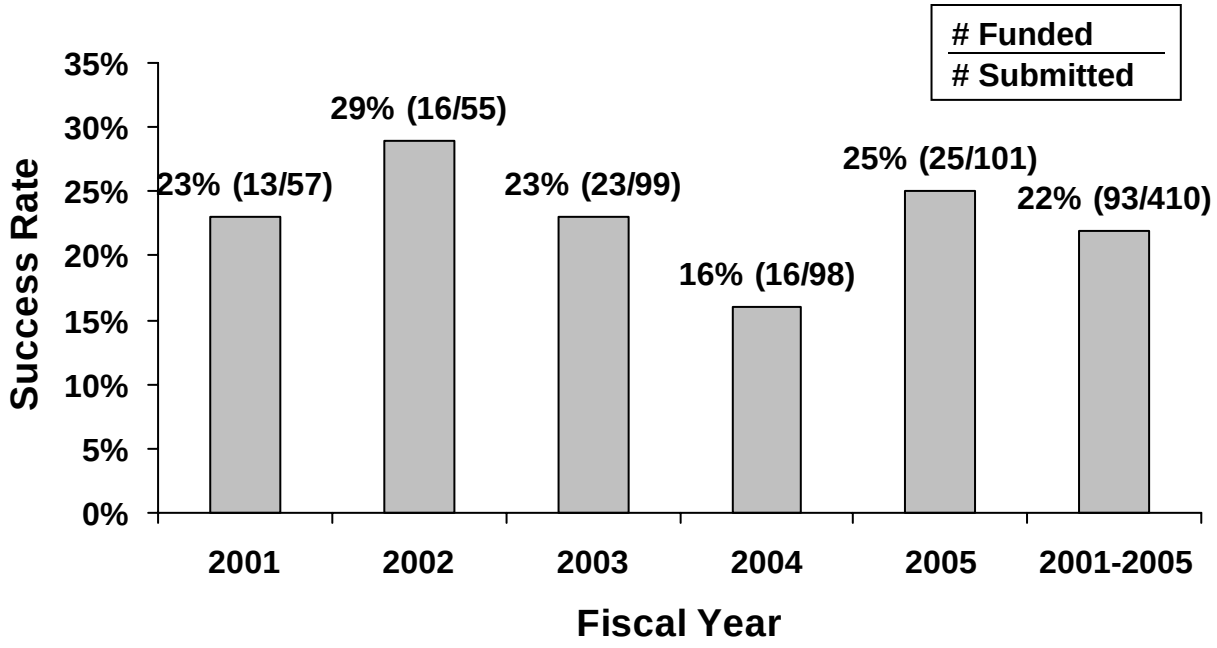

FIGURE 18B: SUCCESS RATE OF APPLICATIONS TO THE PREIMPLANTATION GENETICS AND DEVELOPMENT PROGRAM, BY SUBCATEGORY

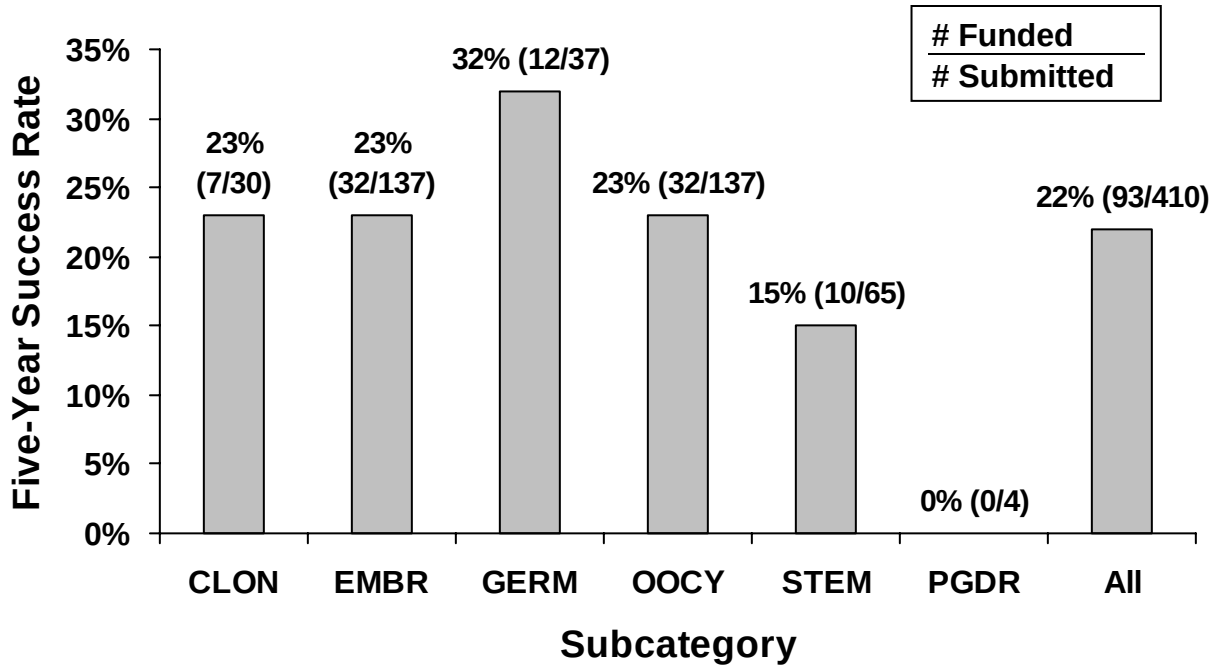

FIGURE 19A: SUCCESS RATE OF APPLICATIONS TO THE MALE REPRODUCTIVE HEALTH PROGRAM, BY FISCAL YEAR

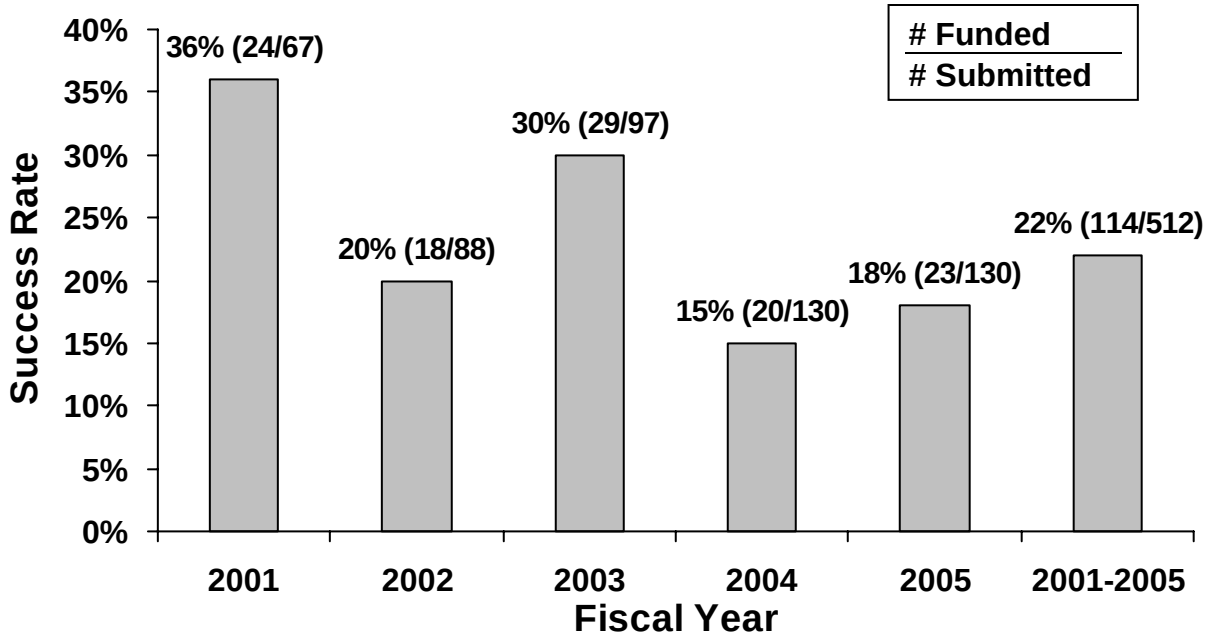

FIGURE 19B: SUCCESS RATE OF APPLICATIONS TO THE MALE REPRODUCTIVE HEALTH PROGRAM, BY SUBCATEGORY

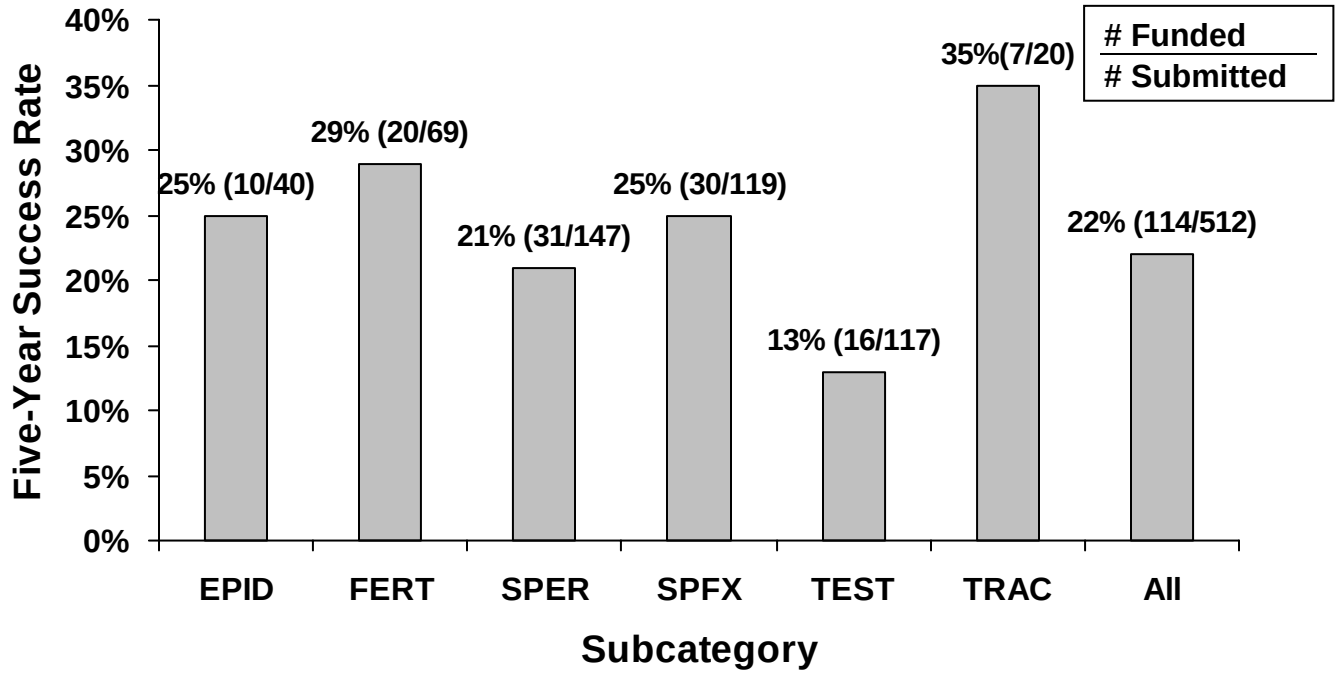

FIGURE 20A: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE GENETICS AND EPIGENETICS/OVARIAN BIOLOGY PROGRAM, BY FISCAL YEAR

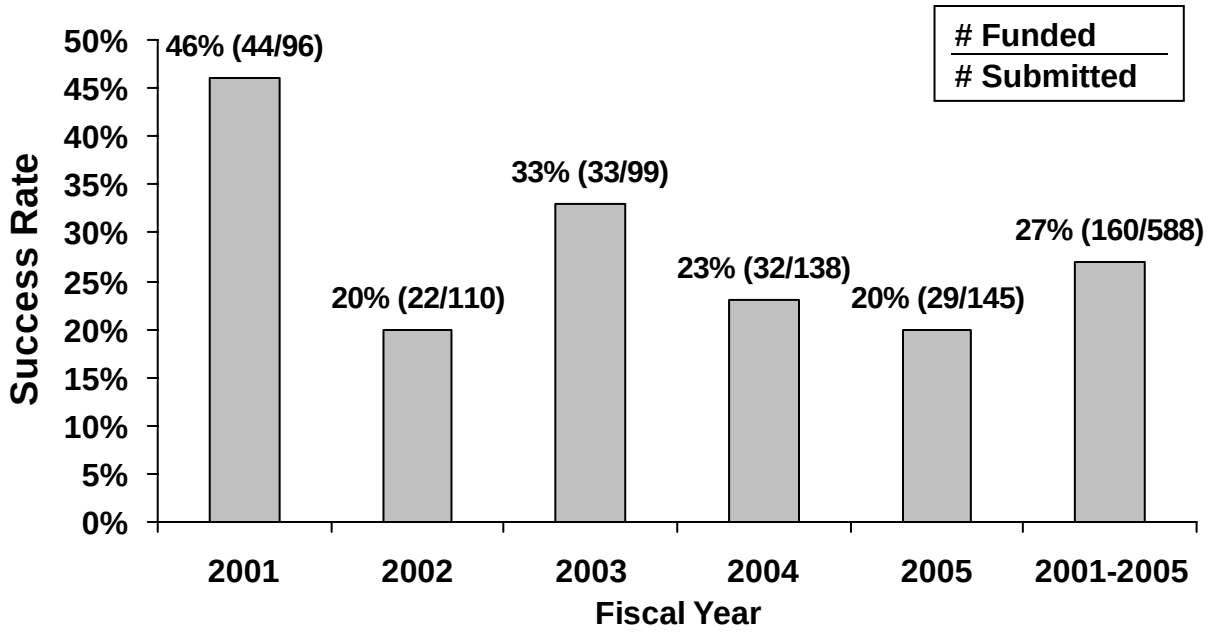

FIGURE 20B: SUCCESS RATE OF APPLICATIONS TO THE REPRODUCTIVE GENETICS AND EPIGENETICS/OVARIAN BIOLOGY PROGRAM, BY SUBCATEGORY

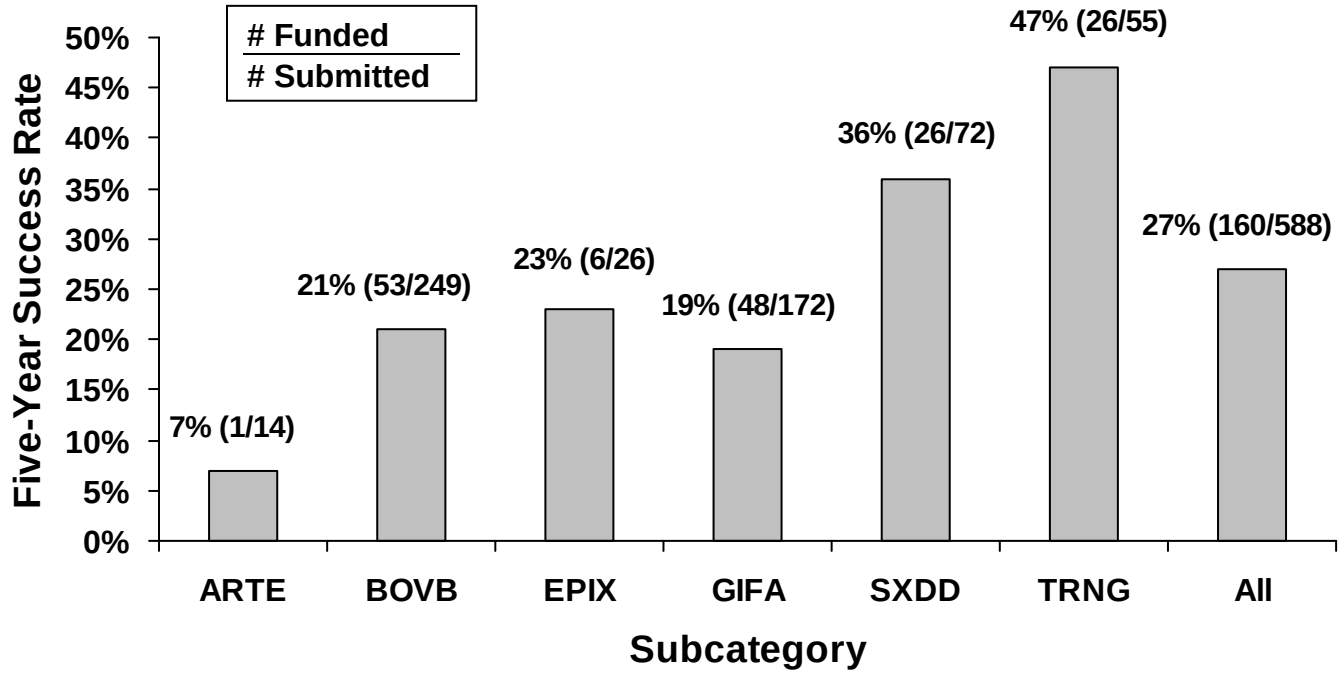

FIGURE 21: RSB FUNDING ON SELECTED DISORDERS, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

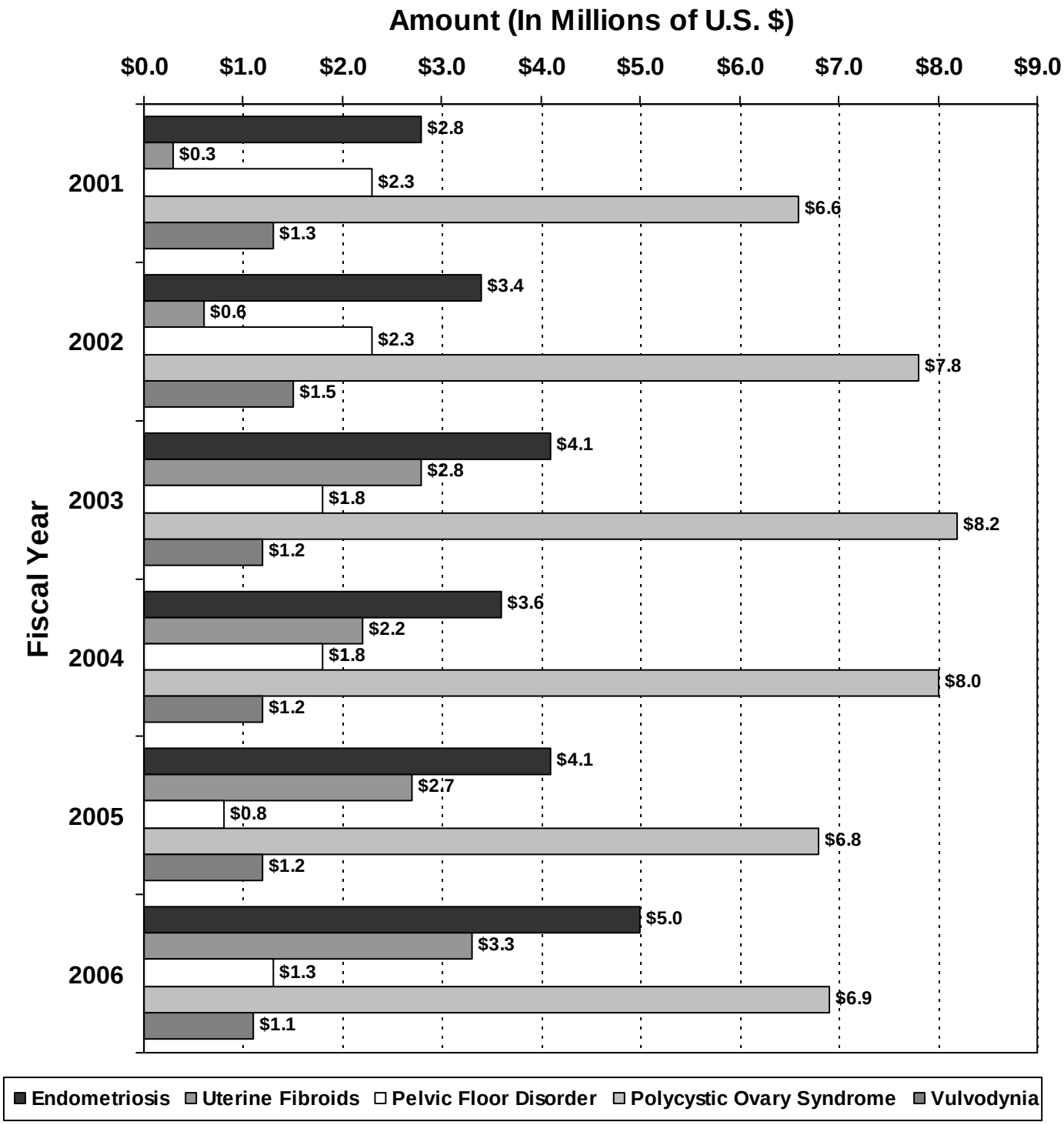

FIGURE 22: NUMBER OF BRANCH PROJECTS ON SELECTED DISORDERS, FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

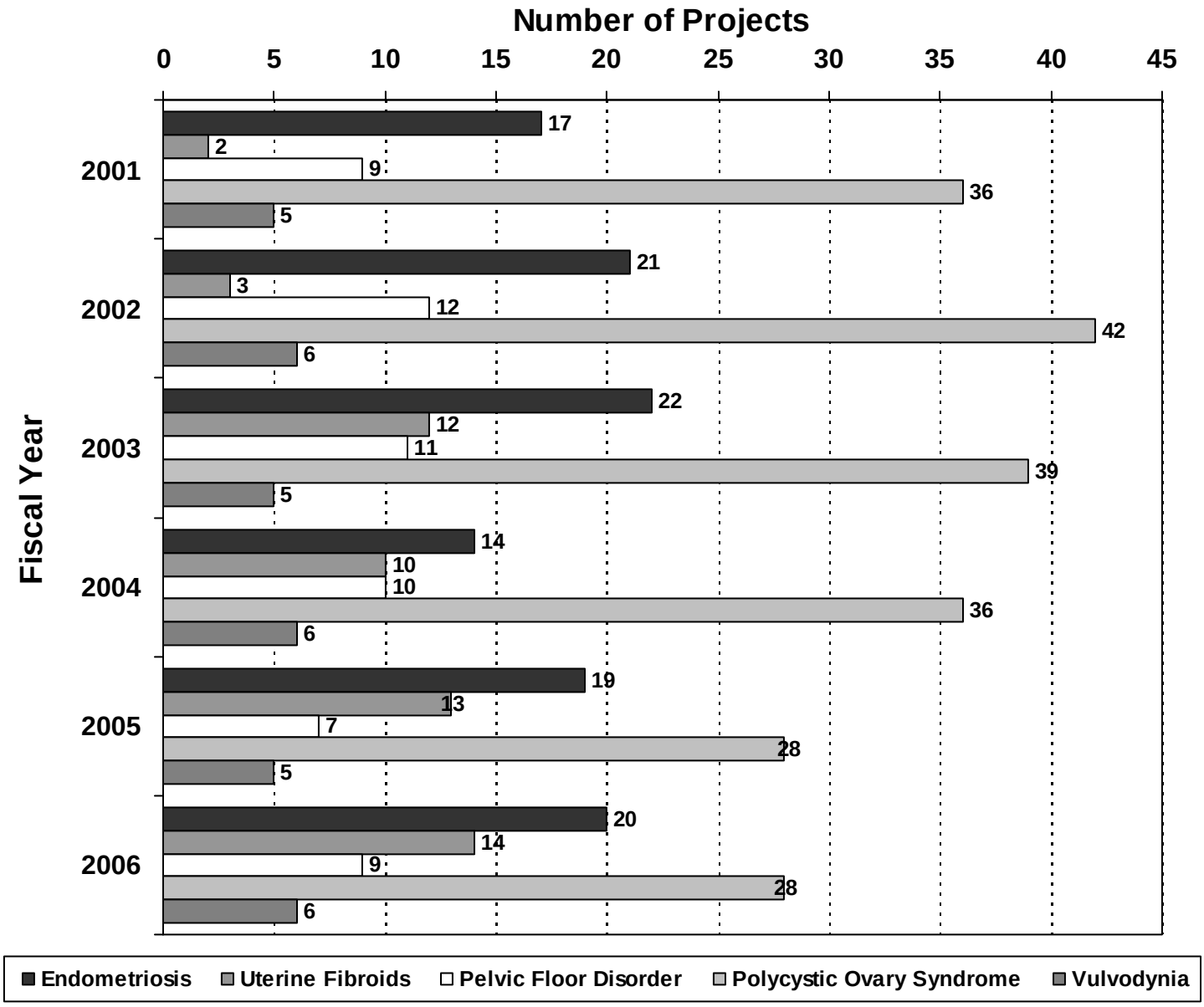

FIGURE 23: SUCCESS RATE OF RSB NEW INVESTIGATORS (SOLICITED RESEARCH PROJECT GRANTS), FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

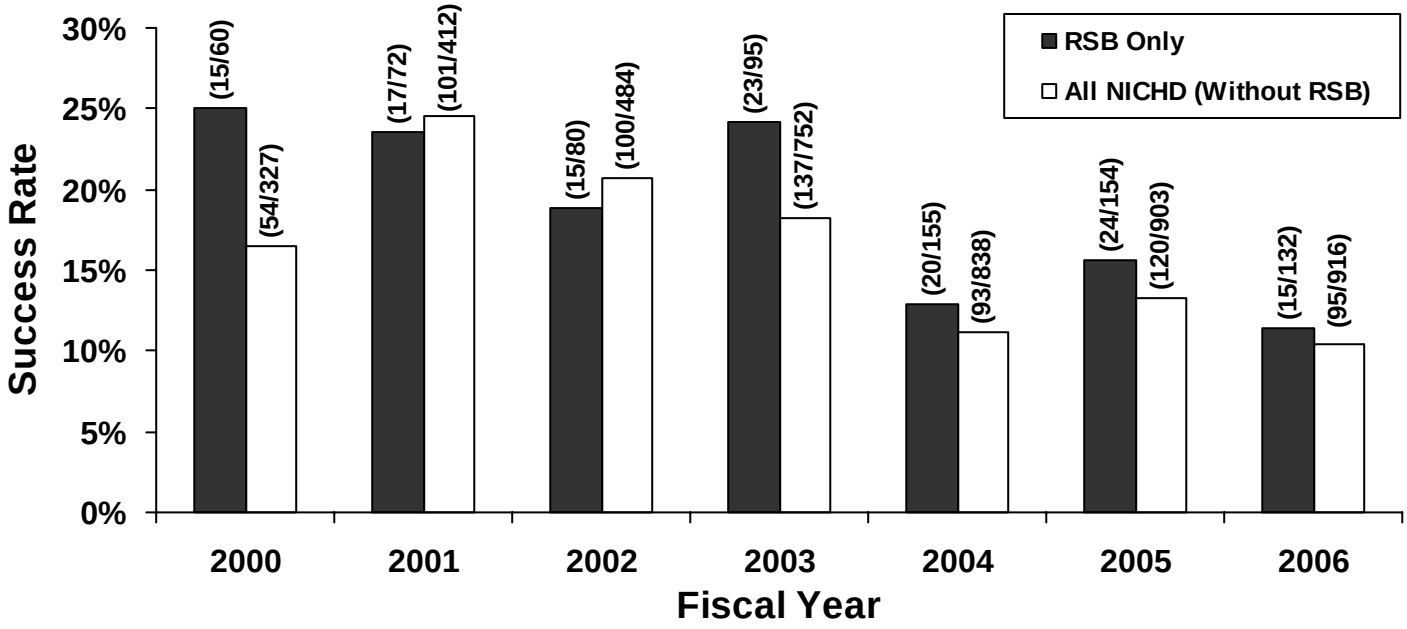

FIGURE 24: SUCCESS RATE OF RSB NEW INVESTIGATORS (UNSOLICITED RESEARCH PROJECT GRANTS), FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

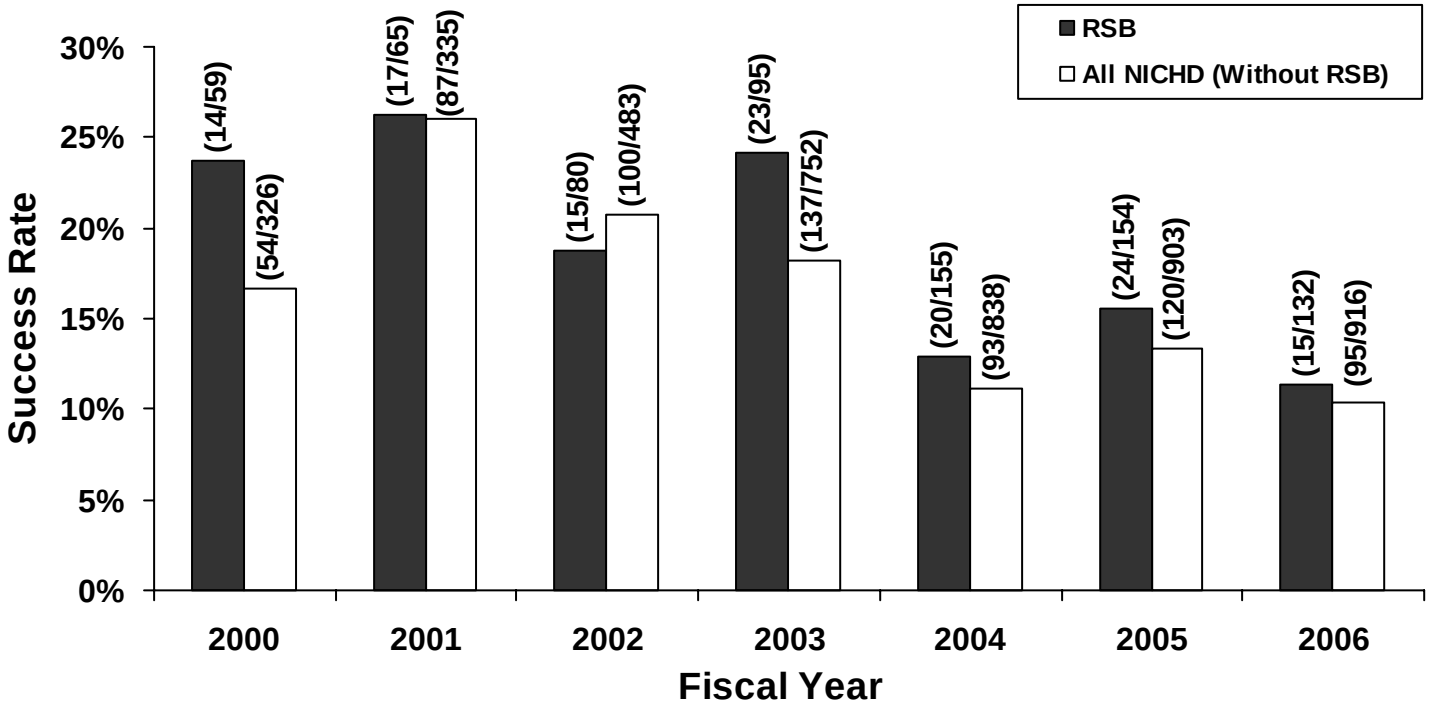

FIGURE 25: SUCCESS RATE OF RSB NEW INVESTIGATORS BY MECHANISM, FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

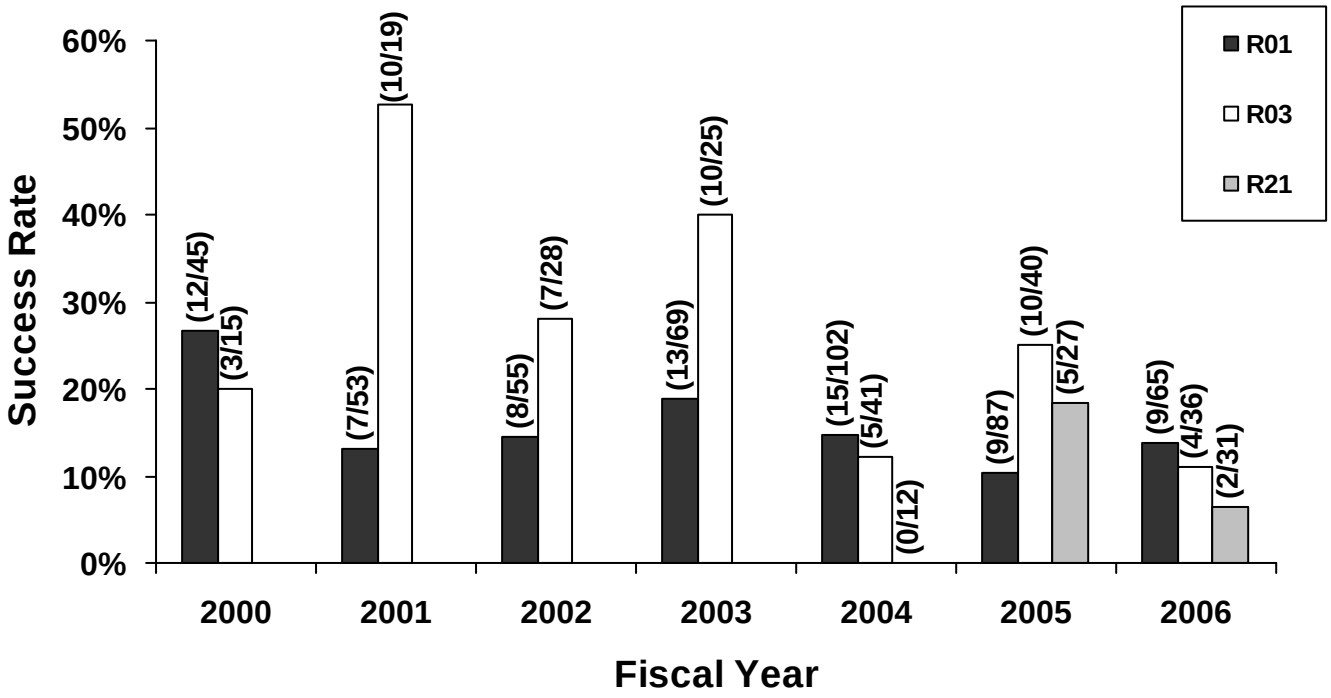

FIGURE 26: SUCCESS RATE OF RSB NEW INVESTIGATORS BY DEGREE, FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

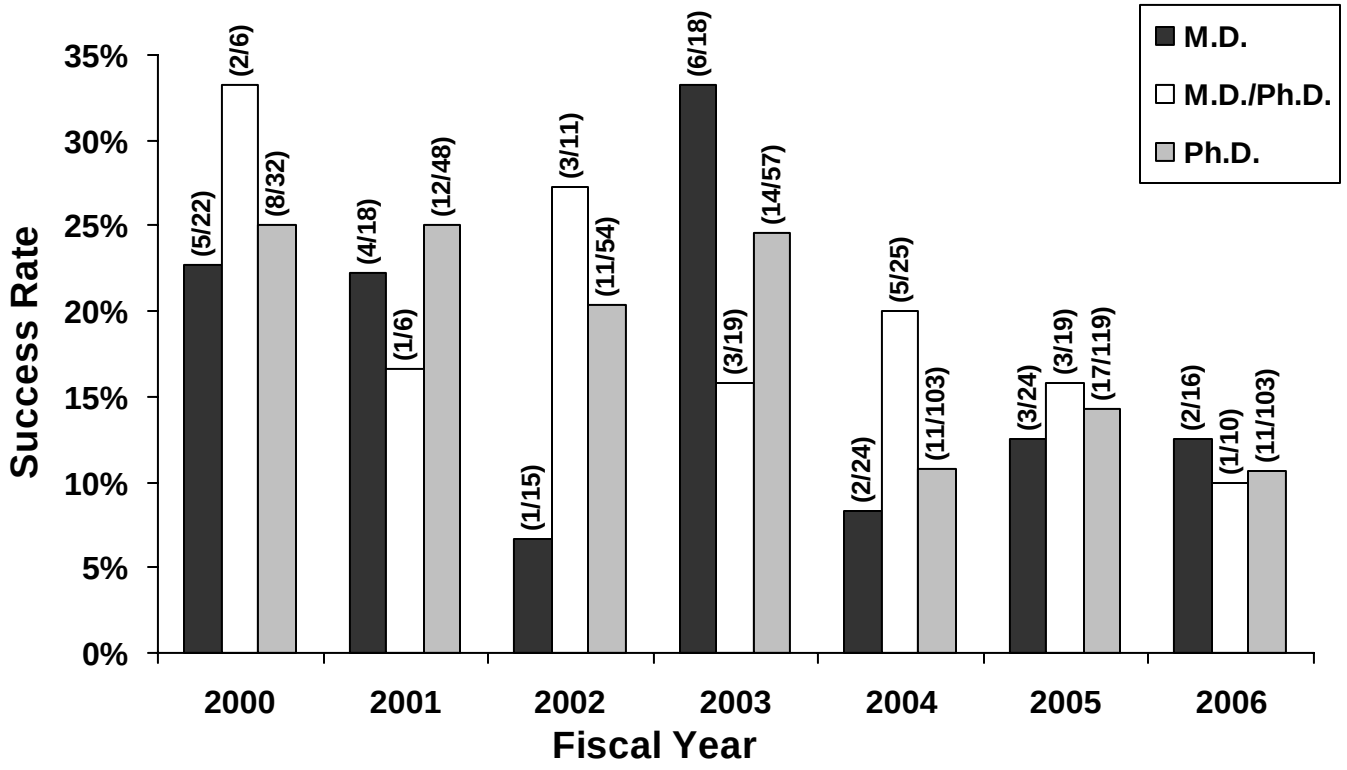

FIGURE 27: SUCCESS RATE OF RSB ESTABLISHED INVESTIGATORS (NEW AND RENEWING), FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

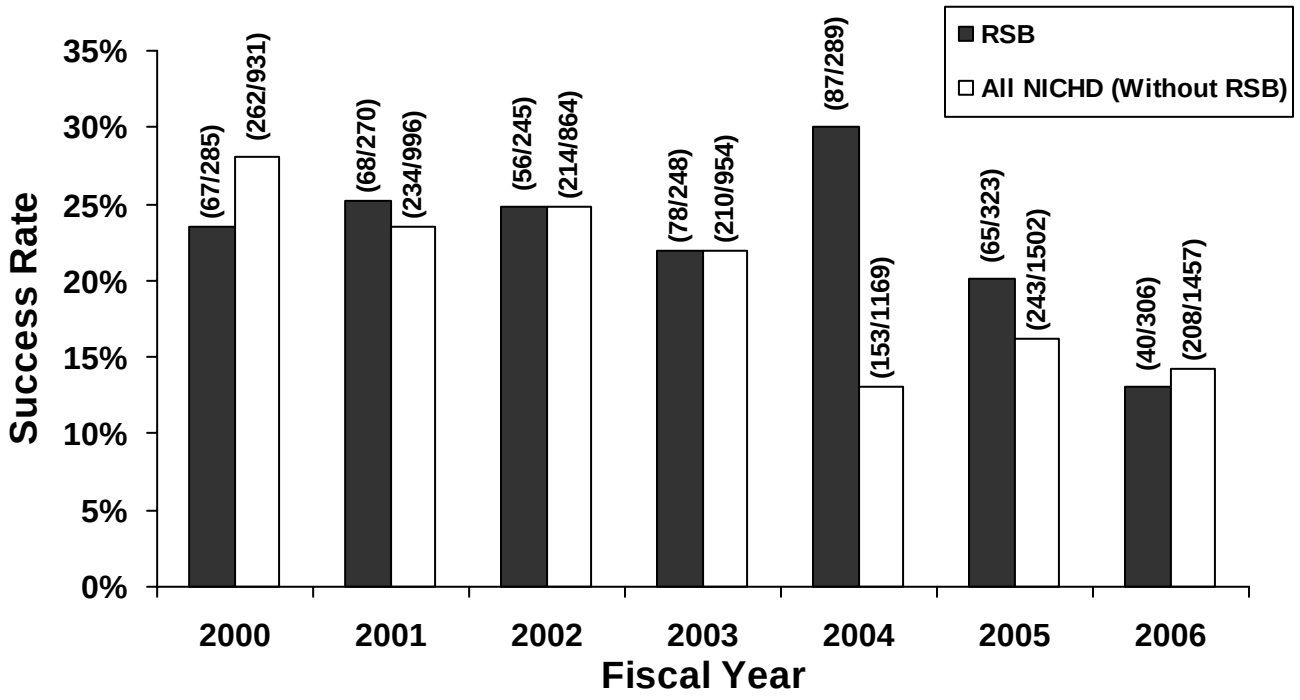

FIGURE 28: SUCCESS RATE OF RSB ESTABLISHED INVESTIGATORS (NEW AND RENEWING) BY MECHANISM, FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

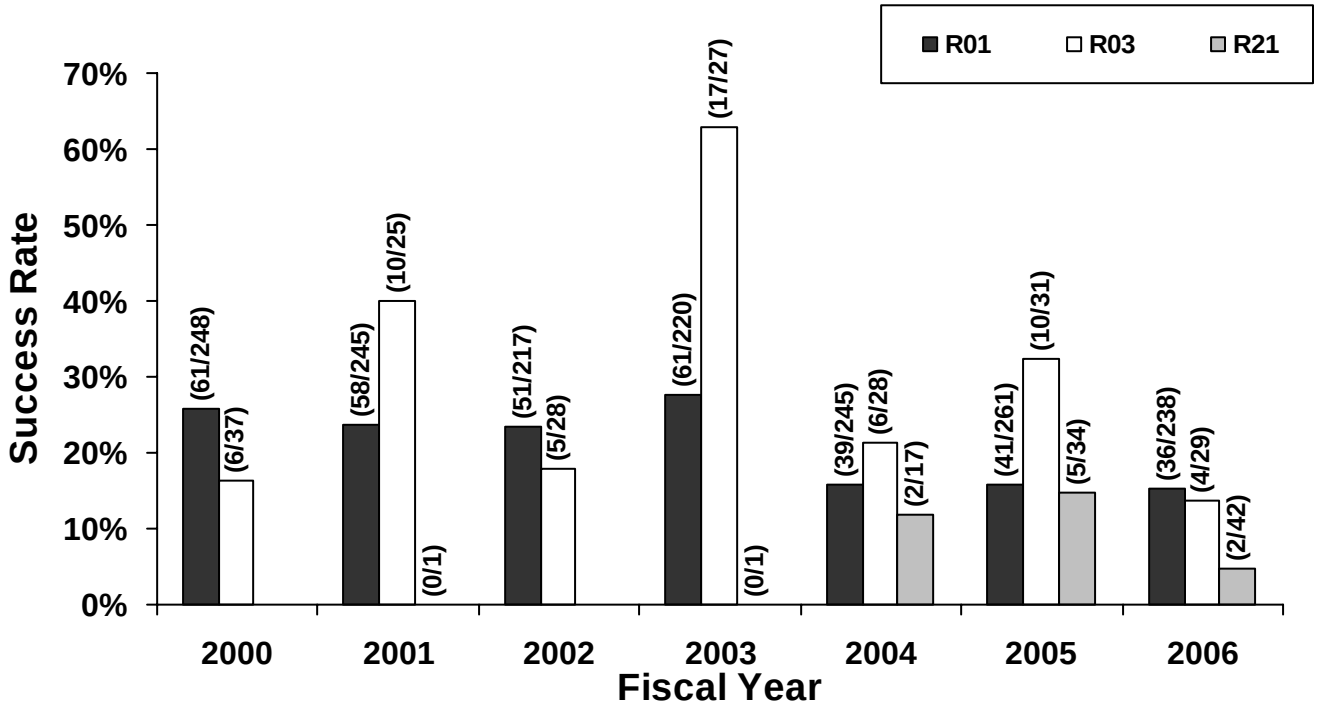

FIGURE 29: SUCCESS RATE OF RSB ESTABLISHED INVESTIGATORS (NEW AND RENEWING) BY DEGREE (M.D., PH.D., M.D./PH.D.), FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

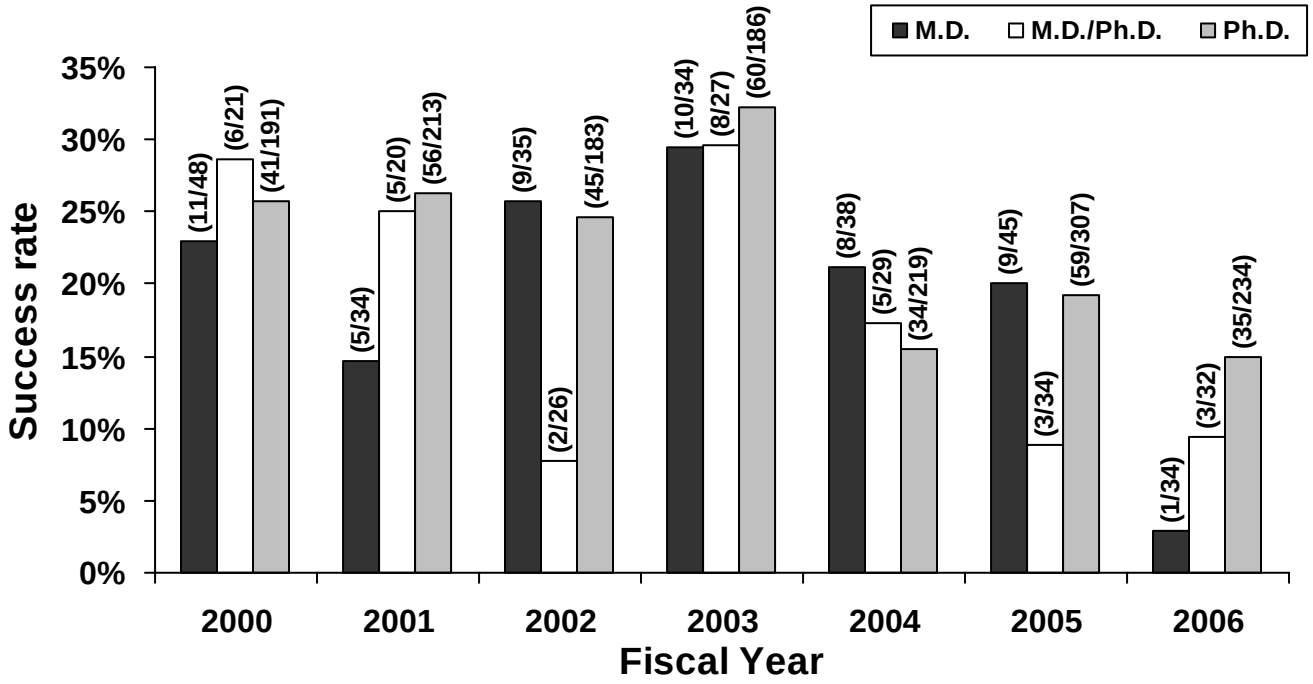

FIGURE 30: SUCCESS RATE OF RSB NEW AND ESTABLISHED INVESTIGATORS—R01S, FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

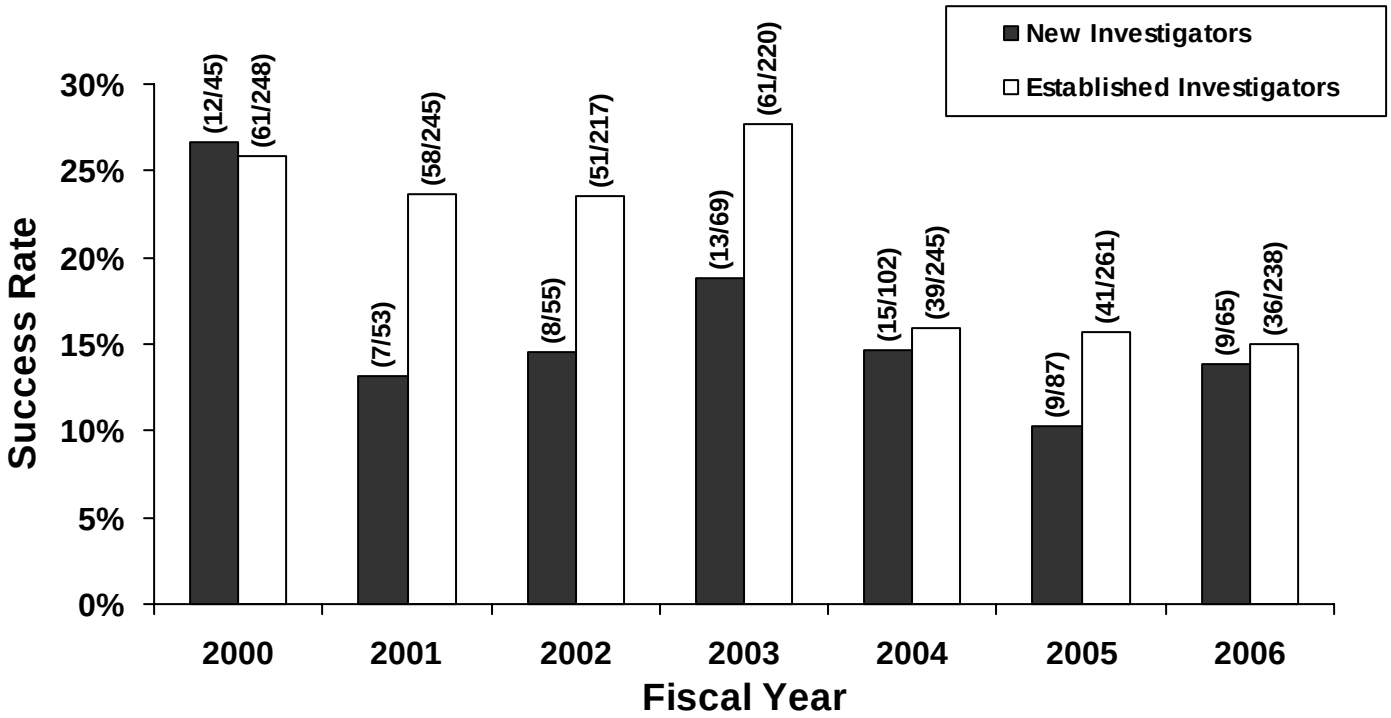

FIGURE 31: SUCCESS RATE OF RSB NEW AND ESTABLISHED INVESTIGATORS—R03S, FISCAL YEAR 2000 THROUGH FISCAL YEAR 2006

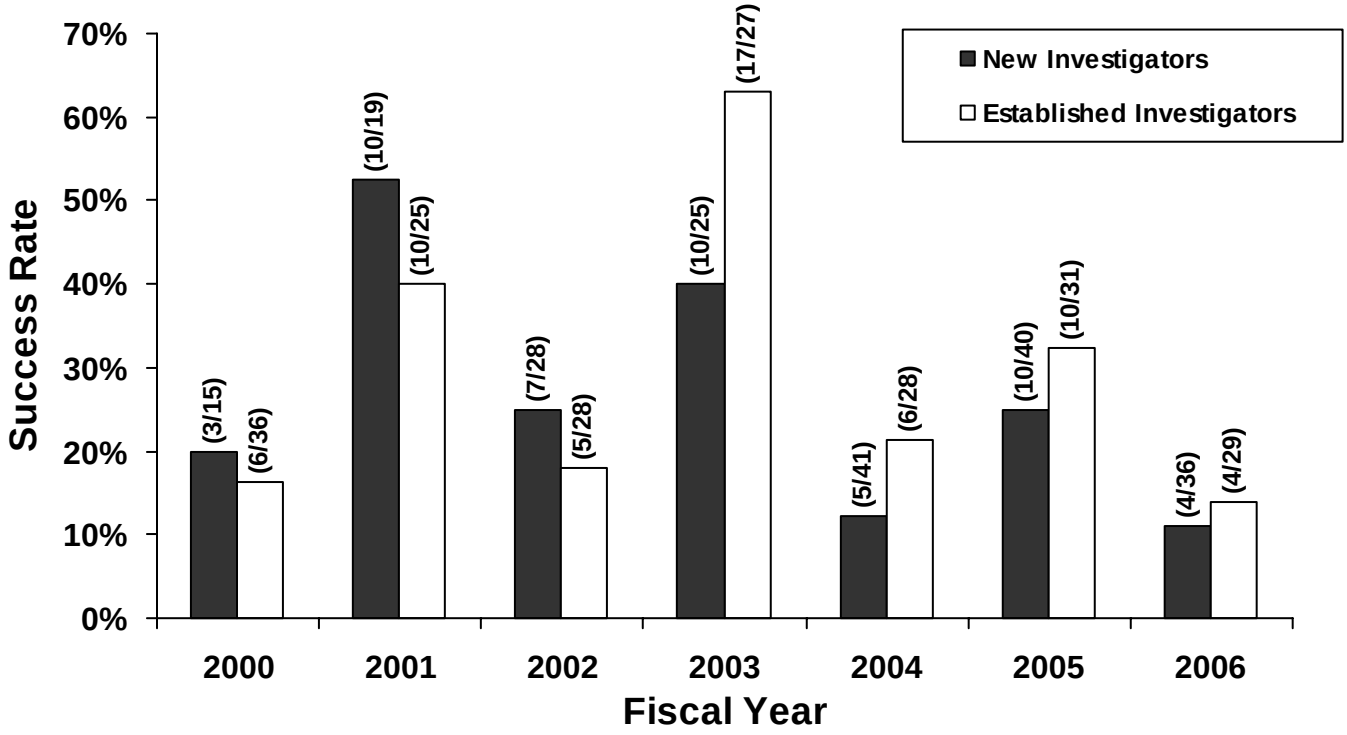

FIGURE 32: STUDY SECTION SUCCESS RATE: REPRODUCTIVE BIOLOGY (REB) AND CELLULAR, MOLECULAR, & INTEGRATIVE REPRODUCTION (CMIR), FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

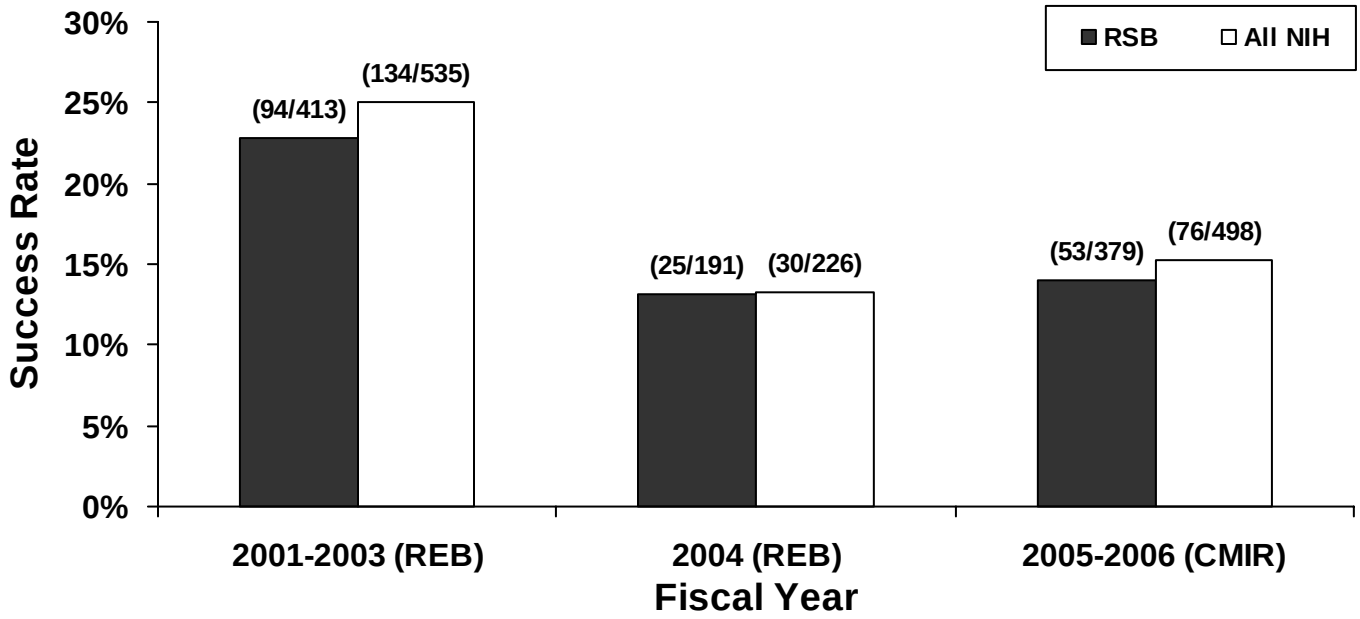

FIGURE 33: STUDY SECTION SUCCESS RATE: REPRODUCTIVE ENDOCRINOLOGY (REN) AND INTEGRATIVE AND CLINICAL ENDOCRINOLOGY AND REPRODUCTION (ICER), FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

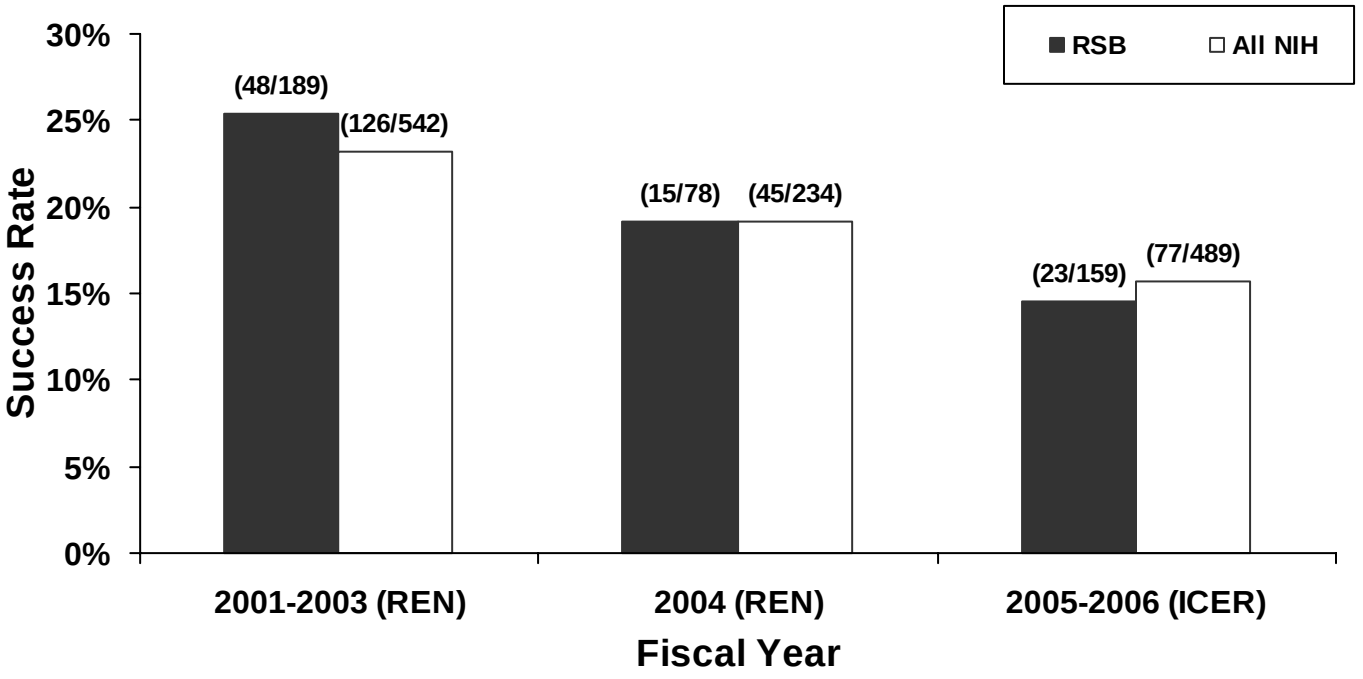

FIGURE 34: STUDY SECTION SUCCESS RATE: HUMAN EMBRYOLOGY (HED-1) AND PREGNANCY AND NEONATOLOGY (PN), FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

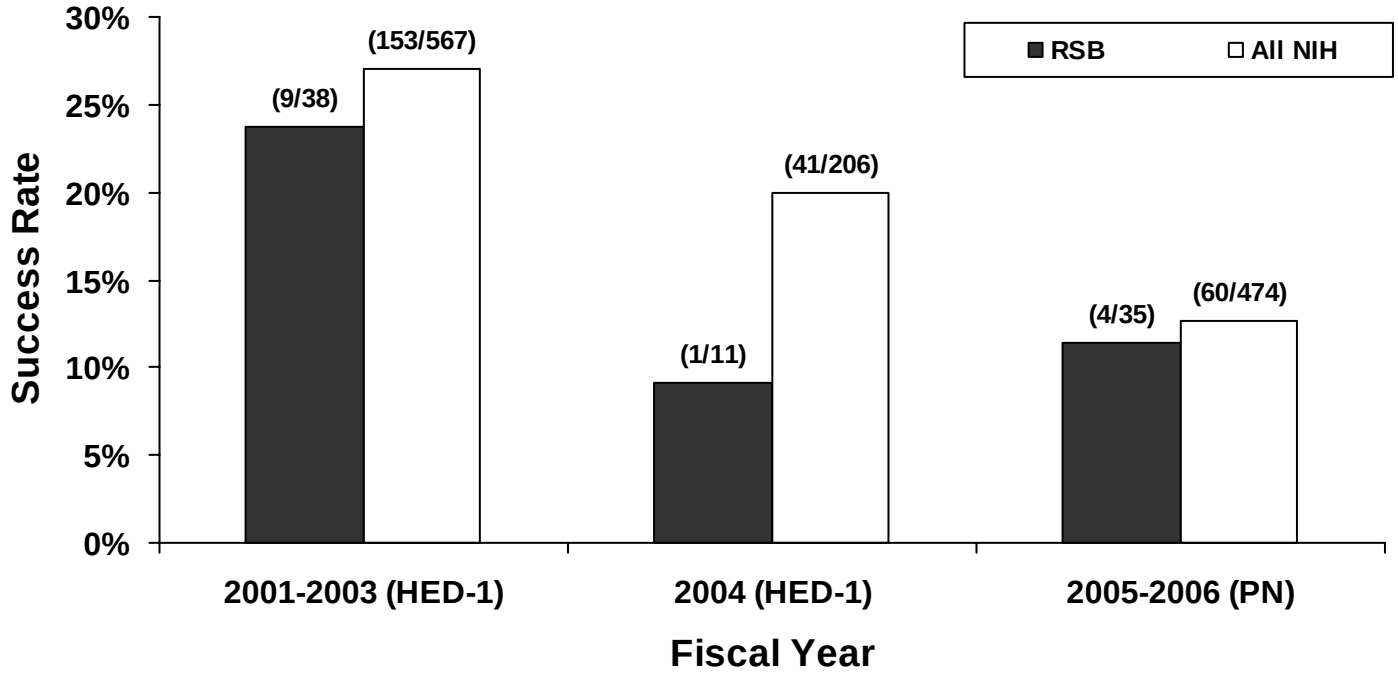

FIGURE 35: STUDY SECTION SUCCESS RATE: HUMAN EMBRYOLOGY (HED-1) AND DEVELOPMENT (DEV-1 & DEV-2), FISCAL YEAR 2001 THROUGH FISCAL YEAR 2006

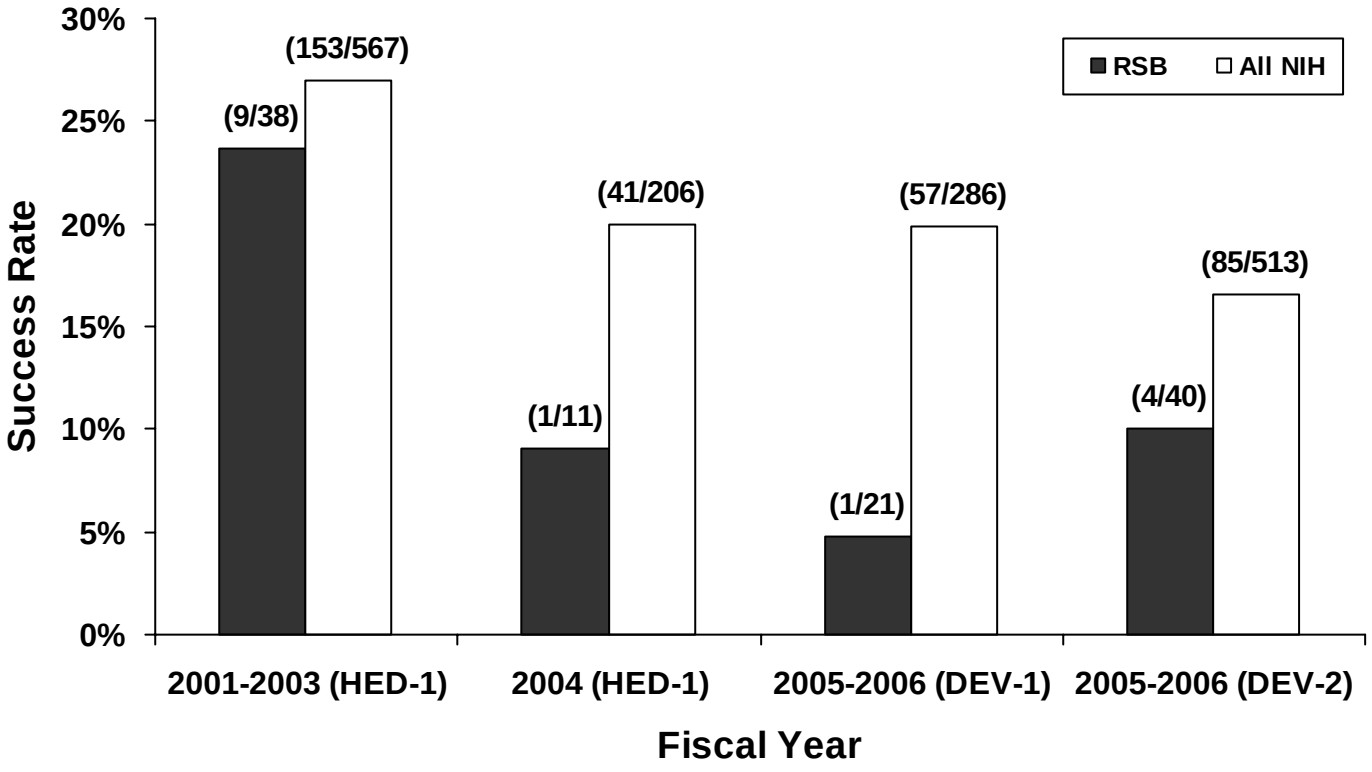

TABLE 2: RSB-FUNDED INDIVIDUAL FELLOWSHIP (F) AWARDS, FISCAL YEAR 2002 THROUGH FISCAL YEAR 2006

Fiscal Year	F31	F32
2002	2	37
2003	4	20
2004	3	10
2005	5	17
2006*	7	18

TABLE 3: RSB-FUNDED CAREER DEVELOPMENT (K) AWARDS, FISCAL YEAR 2002 THROUGH FISCAL YEAR 2006

Fiscal Year	K02	K08	K23	K24	K25
2002	5	5	4	8	1
2003	3	8	6	8	1
2004	5	10	10	8	1
2005	4	9	11	7	0
2006*	3	7	6	4	0

TABLE 4: RSB-FUNDED INSTITUTIONAL FELLOWSHIP (T32) AWARDS, FISCAL YEAR 2002 THROUGH FISCAL YEAR 2006

Fiscal Year	T32 Programs	Pre-Doctoral	Postdoctoral
2002	23	45	59
2003	23	46	59
2004	24	45	61
2005	24	40	62
2006	21	31	56

APPENDIX A: RSB PERSONNEL

Phyllis Leppert, M.D. Ph.D., joined the RSB in 1999 and served as chief until March 2006. She received her Ph.D. from Columbia University and her M.D. from Duke University, and she is a board-certified obstetrician/gynecologist. While with RSB, Dr. Leppert continued to pursue her research interests as a staff scientist in the Reproductive Biology and Medicine Branch, Division of Intramural Research (DIR), NICHD. She was also instrumental in establishing the Clinical Research/Reproductive Scientist Training Program (CREST), an innovative on-line training program for clinicians that was instituted in October 2004.

Louis DePaolo, Ph.D., joined the RSB in 1994 after completing the NIH Grants Associates Program. He became chief of the RSB in July 2006. Dr. DePaolo serves as the research coordinator for both the SCCPRR and the NCPIR. In 1997, he established the Contraception and Infertility Research Loan Repayment Program, which was the first extramural loan repayment program at the NIH. Dr. DePaolo received his undergraduate degree in zoology from Rutgers College and his graduate degree in physiology from the University of Maryland School of Medicine. Prior to coming to NIH, he was an associate professor in the Department of Physiology at the University of Texas Health Science Center, San Antonio, and a member in the Department of Molecular Endocrinology at the Whittier Institute in La Jolla. His research background is in the neuroendocrine control of female reproduction.

Joan Davis, M.D., M.P.H., was with RSB from 2002 to 2005. Dr. Davis was the director of the BIRCWH program and of the NICHD Contraception and Infertility Research Loan Repayment Program. Dr. Davis served as a project team leader for the Roadmap K12 Multidisciplinary Clinical Research Career Development Program.

Charisee Lamar, Ph.D., M.P.H., R.R.T., joined the RSB in May 2005. She directs the Reproductive Neuroendocrinology Program and the BIRCWH program and is developing the Fertility Preservation Program. Dr. Lamar received her undergraduate degree in respiratory therapy from the Medical College of Georgia and has been a registered respiratory therapist since 1989. She earned a doctoral degree in endocrinology from the Medical College of Georgia and received a master's degree in public health from the University of North Carolina, Chapel Hill. Dr. Lamar was a National Cancer Institute, NIH Cancer Prevention Fellow from 1998 to 2002, during which time her research focused on the role of sex hormones and hormone receptors in the risk of breast cancer. Prior to joining the NICHD, she was the centers program director at the National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Estella Parrott, M.D., M.P.H., joined the RSB in 1998. She serves as the program director of the Reproductive Medicine Gynecology Program. She is the program officer for the RMN, the RSDP, the Cooperative Reproductive Science Research Centers at Minority Institutions, the SCOR on Sex and Gender Factors Affecting Women's Health, and the WRHR Career Development Centers. Dr. Parrott earned an undergraduate degree in biology from the City College of New York and a master's degree from the University of Chicago. She received her medical degree from the University of Illinois Medical School, followed by a residency in obstetrics and gynecology. She is board-certified in obstetrics/gynecology and has another

master's degree in public health from the George Washington University. Before assuming her current role, she held positions with the National Institute of Allergy and Infectious Diseases, the Food and Drug Administration, and the Health Resources and Services Administration.

Tracy Rankin, Ph.D., joined the RSB in 2001 and serves as the director for the Male Reproductive Health Program. Dr. Rankin is the project scientist for the National Cooperative Program on Mouse Phenotyping: Developmental and Fertility Defects. 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 Ph.D. in cell biology from Vanderbilt University. She held postdoctoral positions at Tufts University, the Worcester Foundation for Biomedical Research, and at the NIDDK, before joining the RSB. Her research background includes epididymal sperm maturation, spermatogenesis, fertilization, and the structure and function of the mammalian zona pellucida.

Richard Tasca, Ph.D., joined the RSB in 1984 and is the program director for the Pre-implantation Genetics and Development Program. Dr. Tasca is the research coordinator for the NICHD National Cooperative Program on Female Health and Egg Quality. He was formerly associate professor of biology and associate director of the School of Life and Health Sciences at the University of Delaware. He received his undergraduate degree in zoology from the University of Pennsylvania and his graduate degree in mammalian developmental genetics from Temple University. Dr. Tasca's research background is in the genetics, ultrastructure, molecular biology, and nutrient transport mechanisms in pre-implantation embryos.

Susan Taymans, Ph.D., joined the RSB in 1999. Dr. Taymans received her undergraduate degree in biology from the University of Virginia and her Ph.D. in molecular endocrinology from the University of Maryland. She held pre- and postdoctoral Intramural Research Training Awards in the NICHD DIR 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 and Epigenetics and the Basic Ovarian Biology portfolios. Dr. Taymans also manages the RSB's Institutional Training Grants (T32s).

Koji Yoshinaga, Ph.D., joined the RSB in 1978. He is the program director for a portfolio of grants in reproductive endocrinology and immunology. In addition, he serves as the research coordinator for the Cooperative Program on Trophoblast-Maternal Tissue Interactions and for the Cooperative Agreement Conference Grants of the Society for the Study of Reproduction and the Society for Gynecologic Investigation. He serves as director of the RSANET Project and as the research coordinator of the SCCPRR Research Focus Group on Endometrium Function/Dysfunction. He received his bachelor's, master's, 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, and at the ARC Unit of Reproductive Physiology and Biochemistry in Cambridge, England. Dr. Yoshinaga was associate professor of anatomy at Harvard Medical School before joining the RSB. His research background is in implantation and reproductive endocrinology.

APPENDIX B: SELECTED RSB STAFF PUBLICATIONS, 2002-2006

(Staff names appear in **bold**.)

Catherino, WH, Prupas, C, Tsibris, JCM, **Leppert, PC**, Payson, M, Neiman, LK, & Segars, J. (2003). Strategy for elucidating differentially expressed genes in leiomyoma identified by microarray technology. *Fertility and Sterility, 80*, 282-290

Catherino, WH, Salama, A, Potlog-Nahari, C, **Leppert, PC**, Tsibris, JCM, & Segars, J. (2004). Gene expression studies in leiomyomata: new directions for research. *Seminars in Reproduction, 22*, 83-90.

Catherino, WH, **Leppert, PC**, Stenmark, MH, Payson, M, Neiman, LK, & Segars, J. (2004). Dermatopontin expression provides a possible molecular association between fibroids and keloids. *Genes, Chromosomes and Cancer, 40*, 204-217.

Catherino, WH, **Leppert, PC**, & Segars, JH. (2006). The promise and perils of microarray analysis. *American Journal of Obstetrics and Gynecology, 195*, 389-393.

Chu, KC, **Lamar, CA**, & Freeman, HP. (2003). Racial disparities in breast carcinoma survival rates. *Cancer, 97*, 2853-2860.

Coutifaris, C, Myers, ER, Guzick, D, Diamond, M, Carson, S, Legro, R, McGovern, P, Schlaff, W, Carr, B, Steinkampf, M, Silva, S, Vogel, D, & **Leppert. P**. (2004). Histological dating of timed endometrial biopsy tissue is not related to fertility status. *Fertility and Sterility, 82*, 1264-1272.

Dixon, D, **Parrott, EC**, Segars, JH, Olden, K, & Pinn, VW. (In Preparation). *The 2nd NIH International Congress on Advances in Uterine Leiomyoma Research: Conference Summary and Future Recommendations.*

Drzewiecki, G, Tozzi, C, Yu, SY, & **Leppert, PC**. (2005). A dual mechanism of biomechanical change in rat cervix in gestation and postpartum: applied vascular mechanics. *Cardiovascular Engineering: An International Journal, 5*, 187-193.

Lamar, CA, Dorgan, JF, Longcope, C, Stanczyk, FZ, Falk, RT, & Stephenson, HE, Jr. (2003). Serum sex hormones and breast cancer risk factors in postmenopausal women. *Cancer Epidemiology, Biomarkers and Prevention, 12*, 380-383.

Legro, RS, Barnhart, HX, Schlaff, WD, Carr, BR, Diamond, MP, Carson, SA, Steinkampf, MP, Coutifaris, C, McGovern, PG, Cataldo, NA, Gosman, G, Nestler, JE, Giudice, LC, **Leppert, PC**, & Myers, ER. (2007). Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *New England Journal of Medicine, 356*,1-16.

Leppert, PC, Baginski, T, Prupas, C, Catherino, WH, Pletcher, S, & Segars, JH. (2004). Ultrastructure of collagen fibrils in uterine leiomyomas and normal myometrium. *Fertility and Sterility, 82*, 1182-1187.

Leppert, PC, Catherino, WH, & Segars, J. (2006). A new hypothesis about the origin of uterine fibroids based on gene expression profile with microarrays. *American Journal of Obstetrics and Gynecology, 195*, 415-420.

Leppert, PC. (2002). Overview of women's health. *Clinical Obstetrics and Gynecology, 45*(4): 1073-1079.

Leppert, PC. (2004). The changing face of NIH peer review. *Fertility and Sterility, 81*, 279-286.

Leppert, PC, & Peipert, JF. (Eds.) *Primary Care for Women*, second edition. Lippincott, Williams and Wilkins, Philadelphia: 2004.

Leppert, PC, & Turner, M. (Eds.) *Vulvodynia: Towards understanding a pain syndrome—Proceedings from the workshop* (NIH Pub. No. 04-5462) U.S. Government Printing Office, Washington, DC: 2004.

Longui, C, Lemos-Marini, SHV, Figueiredo, B, Mendonca, BB, Castro, M, Liberatore, R, Jr., Watanabe, C, Lancelotti, CLP, Rocha, MN, Melo, MB, Monte, O, Calliari, LEP, Guerra-Junior, G, Baptista, MTM, Sbragia-Neto, L, Latronico, AC, Moreira, A, Tardelli, AMD, Nigri, A, **Taymans, SE**, & Stratakis CA. (2004). Inhibin-alpha subunit (INHA) gene and locus changes in pediatric adrenocortical tumors from TP53 R337H mutation heterozygote carriers. *Journal of Medical Genetics, 41*, 354-359.

Matyakhina, L, Pack, S, Kirschner, LS, Pak, E, Mannan, P, Jaikumar, J, **Taymans, SE**, Sandrini, F, Carney, JA, & Stratakis, CA. (2003). Chromosome 2 (2p16) abnormalities in Carney complex tumors. *Journal of Medical Genetics, 40*, 268-277.

McGovern, PG, Myers, ER, Silva, S, Coutifaris, C, Carson, SA, Legro, R, Schlaff, WD, Carr, BA, Steinkampf, MP, Giudice, LC, **Leppert, PC**, & Diamond, MP. (2004). Absence of secretory endometrium after false positive home urine luteinizing hormone testing. *Fertility and Sterility, 82*, 1273-1277.

Myers, ER, Silva, S, Barnhart, HX, Groben, P, Richardson, MS, Robboy, SJ, **Leppert, P,** Coutifaris, C, & the NICHD National Cooperative Reproductive Medicine Network. (2004). Inter- and intraobserver variability in the histological dating of the endometrium in fertile and infertile women. *Fertility and Sterility, 82*, 1278- 1272.

Payson, M, **Leppert, PC**, & Segars J. (2006). Epidemiology of myomas. Submitted to *Obstetrics and Gynecologic Clinics of North America, 33*, 1-11.

Rankin, TL, Coleman, JS, Epifano, O, Hoodbhoy, T, Turner, SG, Castle, PE, Lee, E, Gore-Langton, R, & Dean, J. (2003). Fertility and taxon-specific sperm binding persist after replacement of mouse "sperm receptors" with human homologues. *Developmental Cell, 5*, 33-43.

Rankin, TL. (2005). Andrology as the medical specialty to focus medical training on men's health? *Journal of Men's Health and Gender, 2*, 45-48.

Yoshinaga, K, & **Parrott, EC**. (Eds.) (2002). Endometriosis: Emerging Research and Intervention Strategies. *Annals of the New York Academy of Sciences, 955*, 1-406.

Yoshinaga, K. (2004). Interface between the endocrine and immune systems in establishment and maintenance of pregnancy; Proceedings from the IX International Congress of Reproductive Immunology. *American Journal Of Reproductive Immunology, 52*(s1), 53-58.

APPENDIX C: PROFESSIONAL STAFF ACTIVITIES

INVITED LECTURES

Phyllis Leppert

- *New Theories of Etiology of Fibroids*, University of Puerto Rico, San Juan, Puerto Rico; January 2002
- *NIH Programs*, Society for Gynecologic Investigation, Trainee Workshop; March 2004
- *Academic Success in an Increasingly Competitive Funding Environment*, Trainee Workshop, the Society for Gynecologic Investigation; March 2005

Louis DePaolo

- *Funding Opportunities at NIH*, 1st Meeting of the Androgen Excess Society, Philadelphia, Pennsylvania; June 2003

Estella Parrott

- *NIH Research Training Opportunities and Loan Repayment Programs*, 8th Annual Research Centers at Minority Institutions, International Symposium on Health Disparities, Honolulu, Hawaii, December 2002
- *Interested in Research? OB-GYN Research Career Development Programs Supported by NIH*, American College of Obstetricians and Gynecologists Annual Clinical Meeting; May 2003, May 2004, & May 2005.
- *Building Infrastructure for Gynecologic Oncology Research Workshop*, Clinical Investigations Branch, National Cancer Institute, Savannah, Georgia; January 2003
- *Current Perinatal Research Initiatives at the NIH*, Annual Perinatal Association Conference, Bethesda, Maryland; October 2003
- *Research Opportunities*, NIH Workshop, Society for Gynecologic Investigation Annual Meeting; March 2004
- *Interdisciplinary Approaches to Women's Health Issues: Training of Future Physician Scientists*, Oregon Health Science University, Beaverton, Oregon; April 2004
- *NIH Research, Training, and Career Development Programs*, Trainee and Career and Junior Faculty Workshop, Society for Gynecologic Investigation, Los Angeles, California; March 2005
- *Advances in Uterine Leiomyoma Research: Program Highlights*, *NICHD Women's Reproductive Health Research Updates*, & *NIH Research and Career Development Programs for OB/GYN Physicians*, National Medical Association Annual Meeting, New York City, New York; July 2005

Richard Tasca

- *Evidence-Based Assisted Reproductive Technologies Workshop*, sponsored by the NICHD RSB, Center for Biologics Evaluation and Research, Food and Drug Administration, & the DHHS Office of Women's Health; September 2002
- *NICHD/NIH Interests in Human Embryonic Stem Cells*, Frontiers in Human Embryonic Stem Cell Workshop, University of Pittsburgh, Pittsburgh, Pennsylvania; May 2003 & May 2004

LIAISON ACTIVITIES

Phyllis Leppert

ACOG Genetics Committee, 2001-2005; NICHD Contraceptive Microbicide Subcommittee; NICHD Data Safety Monitoring Committee, 2002-2006; NICHD Fertility and Early Pregnancy Working Group, National Children's Study; DHHS Assisted Reproductive Technology Working Group, 2001-2003; Quarterly News Briefs, American Society of Reproductive Medicine Newsletter, 2001-2005; Society for Gynecologic Investigation Program Committee, 2000-2005; Trans-NIH Endocrinology, Metabolism and Reproductive Sciences Integrated Review Group Steering Committee; NICHD Contraception and Infertility Research Loan Repayment Program Selection Committee; NIH Roadmap Translational Science Roadmap Committee, 2003; NIH Rapid Access to Interventional Development Committee, 2004-2006; National Coalition for Oversight of Assisted Reproductive Technologies, 2003-2006; CONRAD, 2000-2006.

Louis De Paolo

Chair, NIH Loan Repayment Program Oversight Panel, 2005-present; NIH Biodefense Research Coordinating Committee, 2004-present; NIH Clinical Research Network Roadmap Workgroup, 2003; NIH Staff Training in Extramural Programs Committee, 2001-2004; Trans-NIH Endocrine Group, NICHD Obesity Working Group, 2003-present; NICHD Organizing Committee for Workshop on Reproduction and the Fragile X Premutation, 2004- 2005; Endocrine Society, Student Affairs Committee, 2000-2003; Endocrine Society Research Affairs Committee, 2001-2004; Society for the Study of Reproduction, Education Committee, 2002-2005.

Charisee Lamar

Trans-NIH/OD Population Tracking Committee, 2003-2004; Trans-NIH/OD ORWH Coordinating Committee for Research on Women's Health, 2003-2005; ORWH Specialized Centers for Research on Sex and Gender Factors Affecting Women's Health Advisory Committee, 2002-present; Building Interdisciplinary Research Careers in Women's Health Committee, 2003-present; National Center for Research Resources Clinical Research Education and Career Development Award Advisory Committee, 2002-2005; Trans-NIH Special Populations Forum, 2003-2005.

Estella Parrott

NIH Liaison, Gynecologic Practice Committee, American College of Obstetricians and Gynecologists, 2000-2005; NIH Liaison, Preconception Care Initiative Workgroup, American College of Obstetricians and Gynecologists, 2005; NICHD Advisory Group on Health Disparities, 1999-2003; NICHD Research Supplements for Underrepresented Minorities Review

Committee, 2000-2005; NIH Coordinating Committee on Research on Women's Health, 1999-2005; NIH ORWH Coordinating Committee on Research on Women's Health Career Development Subcommittee, 2000-2005; NIH Extramural Associates Advisory Board, 1999-2002; ORWH NIH *Ad Hoc* Working Group Review Committee for the Research Enhancement Awards Program, 1998-2005; ORWH Task Force Recruitment and Retention in Clinical Trials Workshop, 2003; NICHD Obesity Committee, 2003-2005; Trans-NIH *Ad Hoc* Menopause Research Working Group, 2005; Trans-NIH *Ad Hoc* Working Group on Uterine Leiomyoma Research, 2005.

Tracy Rankin

NICHD representative on the Urology Subcommittee of the Kidney, Urologic, and Hematologic Diseases Interagency Coordinating Committee; NICHD representative to the Trans-NIH Mouse Genomics and Genetics Resources Coordinating Group.

Richard Tasca

NIH Stem Cell Task Force; NIH Stem Cell Implementation Committee; American Society for Cell Biology Local Arrangements Committee, 2004; NIH representative to the National Coalition for the Oversight of Assisted Reproductive Technologies; Briefings of HHS staff, congressional staff, South Korean delegation, and German delegation on stem cell research, 2003-2005.

Susan Taymans

NICHD Training Policy Committee, 2002-present; NICHD project scientist for Genitourinary Developmental Mouse Atlas Project, 2004-present.

APPENDIX D: RSB-SUPPORTED CONFERENCES AND WORKSHOPS

- *Evidence-Based Assisted Reproductive Technologies Workshop*, Co-sponsored with the Center for Biologics Evaluation and Research, Food and Drug Administration, & DHHS Office of Women's Health; Bethesda, Maryland; September 18-19, 2002
- *Emerging Technologies for the Study of Reproductive Neuroendocrinology*, Bethesda, Maryland; October 24-25, 2002
- *WRHR Scholars' Research Symposium*, Bethesda, Maryland; March 31-April 1, 2003
- *Vulvodynia: Toward Understanding a Pain Syndrome*, Washington, DC; April 14-15, 2003
- *Role of Genomic Imprinting, Confined Placental Mosaicism and Uniparental Disomy in Fetal Growth and Beyond*, Potomac, Maryland; May 2003
- *Biennial SCCPRR Research Meeting*, Bethesda, Maryland; May 13-14, 2003
- *The WRHR Program: Transition to Independence for Physician Scientists*, Bethesda, Maryland; October 27, 2003
- *Reproduction 2003*, Bethesda, Maryland; November 10, 2003
- *Crossing Over: Genetics and Reproductive Biology*, Bethesda, Maryland; October 7-8, 2004
- *USDA-NIH Workshop on Advantages of Agriculturally Important Domestic Species as Biomedical Models*, East Lansing, Michigan; October 29-31, 2004
- *The 2nd NIH International Congress on Advances in Uterine Leiomyoma Research*, Bethesda, Maryland; February 24-25, 2005
- *Health Disparities in Infertility*, Bethesda, Maryland; March 10-11, 2005
- *Biennial SCCPRR Research Meeting*, Chicago, Illinois; April 14-15, 2005
- *WRHR Scholars' Research Symposium*, Cincinnati, Ohio; May 16-17, 2005
- *Molecular Mechanisms of Fertilization*, Worcester, Massachusetts; July 16-17, 2005
- *Infertility Treatment and Adverse Pregnancy Outcomes*, Washington, DC; September 12-13, 2005
- *New Horizons in GnRH Research*, Bethesda, Maryland; November 10-11, 2005

APPENDIX E: RSB-SPONSORED FUNDING OPPORTUNITY ANNOUNCEMENTS AND SOLICITATIONS

REQUESTS FOR APPLICATIONS (RFA)

- RFA-HD-02-018—Female Health and Egg Quality
- RFA-HD-02-029—Specialized Cooperative Centers Program in Reproduction Research (SCCPRR)
- RFA-HD-03-005—Leiomyomata Uteri: Basic Science and Translational Research
- RFA-HD-04-003—SCCPRR
- RFA-HD-04-014—Women's Reproductive Health Research Career Development Centers
- RFA-HD-04-030—SCCPRR
- RFA-HD-05-040—Male Reproductive Health Research Career Development Program
- RFA-HD-05-055—Specialized Cooperative Centers Program in Reproduction and Infertility Research (SCCPIR)
- RFA-HD-06-005---SCCPIR
- RFA HD-06-008---Cooperative Multicenter Reproductive Medicine Network
- RFA HD-06-017---Cooperative Reproductive Science Research Centers at Minority Institutions

PROGRAM ANNOUNCEMENTS (PA/PAR)

- PAR-02-143—Development of Cell-Selective Tools for Studies of the Bladder, Prostate, and Genitourinary Tract (with NIDDK)
- PA-03-079—Emerging Technologies for the Study of Reproductive Neuroendocrinology
- PA-03-116—Transmission of Human Immunodeficiency Virus in Semen (with NIDDK)
- PA-04-049—Reproductive Genetics and Epigenetics
- PA-04-146—Pilot and Feasibility Program in Urology (with NIDDK)
- PA-04-056---Endometrial Cell Function
- PAR-04-042—Murine Atlas of Genitourinary Development (with NIDDK)
- PA-06-032---Vulvodynia – Systematic Epidemiologic, Etiologic or Therapeutic Studies (R01)
- PA-06-346—Reproductive Genetics and Epigenetics (R21)
- PA-06-347—Reproductive Genetics and Epigenetics (R03)

NOTICES (NOT)

- NOT-HD-03-005—Administrative Supplements for Human Embryonic Stem Cell Research
- NOT-HD-05-011—Administrative Supplements for Human Embryonic Stem Cell Research

APPENDIX F: EXPERT PANEL

William Bremner, M.D., Ph.D.
University of Washington
School of Medicine
Seattle, WA 98195

William Crowley, M.D.
Harvard Medical School
Massachusetts General Hospital
Boston, MA 02114

Deborah Driscoll, M.D.
University of Pennsylvania Hospital
Department of Obstetrics and Gynecology
Philadelphia, PA 19104

John Eppig, Ph.D.
The Jackson Laboratory
Bar Harbor, ME 04609

Linda Giudice, M.D., Ph.D.
University of California, San Francisco
Department of Obstetrics, Gynecology
and Reproductive Sciences
San Francisco, CA 94143

Judith Hall, M.D.
University of British Columbia
Department of Pediatrics
Vancouver, British Columbia V6H 3V4
(NACHHD Council Representative)

Cassandra Henderson, M.D.
Our Lady of Mercy Medical Center
Bronx, NY 10466

Joseph Isaacs, M.S.P.H.
Resolve, Inc: The National Infertility
Association
Bethesda, MD 20814

David McClay, Ph.D.
Duke University
Department of Biology
Durham, NC 27708

Janet Rossant, Ph.D.
Hospital for Sick Children
Toronto, Ontario M5G 1X8

M. Susan Smith, Ph.D.
Oregon National Primate Research Center
Oregon Health and Science University
Beaverton, OR 97006

Teresa Woodruff, Ph.D.
Northwestern University
Department of Neurobiology and
Physiology
Evanston, IL 60208