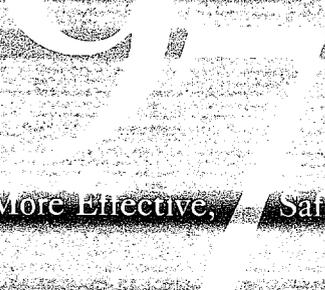


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THE CONRAD PROGRAM

N I N E E N A N N U A L R E P O R T



Making Progress toward More Effective, Safe, and Acceptable Contraceptives

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HIGHLIGHTS

CONRAD'S ACHIEVEMENTS IN 1997

Research takes time—years and sometimes decades—so it is particularly noteworthy that in 1997 several CONRAD-supported activities advanced to the next stage of research and development. Four major achievements are highlighted here.

FemCap™

Results of the Phase II/III clinical trial on this new cervical cap, which was completed in 1997, showed that efficacy is similar to existing barrier methods. It is expected that the U.S. Food and Drug Administration (FDA) will approve FemCap™ in 1998.

Easy Fit Diaphragm

As a result of 1997 research, the Easy Fit diaphragm has reached the stage where postcoital studies can take place. This newly designed diaphragm was developed by the Program for Appropriate Technology in Health (PATH) in collaboration with CONRAD using a novel user-perspective approach.

Q2

Research on this CONRAD-owned polymer base for spermicides and microbicides progressed through sufficient preclinical evaluation in 1997 to allow human safety studies to begin.

Male Methods

Work continued on dose-finding studies on male methods of hormonal contraception using various combinations of progestins and androgens that result in infertility but have minimal adverse effects. A clinical trial combining Norplant®II with a testosterone patch is underway.

About CONRAD

SINCE 1986, THE CONRAD PROGRAM has supported the development of better, safer, and more acceptable methods of fertility regulation that are suitable for use in developing countries. Established under a cooperative agreement with the U.S. Agency for International Development (USAID) and the Eastern Virginia Medical School in Norfolk, Virginia, CONRAD places priority on moving promising leads through Phase I and II clinical trials. Research supported by CONRAD is conducted at its intramural laboratories and clinical facilities in Norfolk and by investigators at public and private sector research institutions in the United States and abroad.

USAID, the National Institutes of Health (NIH), and the Centers for Disease Control and Prevention (CDC) are the primary sources of CONRAD's federal funding. Because of the link among HIV/AIDS prevention, contraceptive practice, and reproductive health, NIH supports research on sexually transmitted diseases (STDs)—in particular, the mechanisms of HIV transmission and the establishment of relevant animal models—and CDC provides support for epidemiological studies. NIH also provides funding for clinical and related preclinical studies of potential new contraceptive products for women or men and cosponsored a recent workshop on formulations of vaginal microbicides and spermicides.

Private foundations also play a role in support of CONRAD activities, particularly in contraceptive development. The Andrew W. Mellon Foundation and The Rockefeller Foundation have been instrumental in providing early-stage contraceptive development funding through CONRAD's Consortium for Industrial Collaboration in Contraceptive Research (CICCR).

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Small Grants and Twinning Program

In 1993, The Andrew W. Mellon Foundation awarded CONRAD a three-year grant to fund small research projects and fellowship training in contraceptive research. The Mellon grant allowed CONRAD to fund projects on more fundamental research and in countries proscribed by USAID. In 1994, the Mellon Foundation asked CONRAD to manage an expansion of its ongoing twinning program between investigators at the Mellon Reproductive Biology Centers and selected centers outside the United States. Recently, another three-year grant was awarded for continued support of CONRAD's small grants program and further expansion of the twinning program. The small grants program supports innovative, high-risk research that may result in new contraceptive leads. The twinning program's goals are to promote research relevant to contraceptive development and build partnerships with centers of excellence in developing countries.

Consortium for Industrial Collaboration in Contraceptive Research

CONRAD launched CICCRR in October 1995 with funding from The Rockefeller Foundation and additional contributions from The Andrew W. Mellon Foundation, The William and Flora Hewlett Foundation, and others. CICCRR has as its primary goal the revitalization of industry's commitment to developing new contraceptive products. Needed innovation has been stymied by a long and costly research and development process and by perceptions among pharmaceutical manufacturers of multiple barriers to entering the market with a new contraceptive.

CICCRR specifically supports the development of new contraceptive agents that address the needs and perspectives of women, as articulated during the 1994 U.N. International Conference on Population and Development in Cairo, Egypt. This conference underscored the need for women and men to be informed about and have access to safe, effective, and affordable methods of family planning and other reproductive health care services.

CICCR identifies leads under investigation by not-for-profit organizations in developed and developing countries that might lead to new methods primarily in the following areas of research:

- contraceptive methods for men;
- vaginal methods that prevent pregnancy and STD transmission; and
- monthly regimens, which could be postcoital, anti-implantation, or menses-inducers.

CICCR provides funds to researchers through three different mechanisms.

- The feasibility projects program is designed to support innovative, high-risk research relevant to the Consortium's objectives. The program seeks to encourage interdisciplinary research, mobilize intellectual capacity outside the field, and recruit new investigators into the field. This initial funding helps bring these projects to the stage where an industrial partner may become interested in collaborating on further development.
- Matching funds with industry fosters collaboration between not-for-profit sector research institutions and industry. Support is limited to the early stages of drug development through Phase II clinical trials. This support may also be used to explore the feasibility of commercial development of scientific findings from these trials, obtain patent protection for their invention, and seek out industrial partners and foster collaboration with such partners.
- Twinning funds are provided only by The Andrew W. Mellon Foundation for collaborations between Mellon-supported Reproductive Biology Centers in the United States and twinning centers in developing countries.

Contraceptive Research Activities

The need to develop new contraceptives for use in developing countries is more urgent than ever. At the same time, researchers worldwide have made significant advances in understanding the underlying scientific mechanisms of contraception. Mindful of the need for new contraceptives and taking advantage of scientific advances, CONRAD has focused its work in five priority areas where it can make a difference:

1. Microbicides to prevent the transmission of HIV/AIDS and other STDs

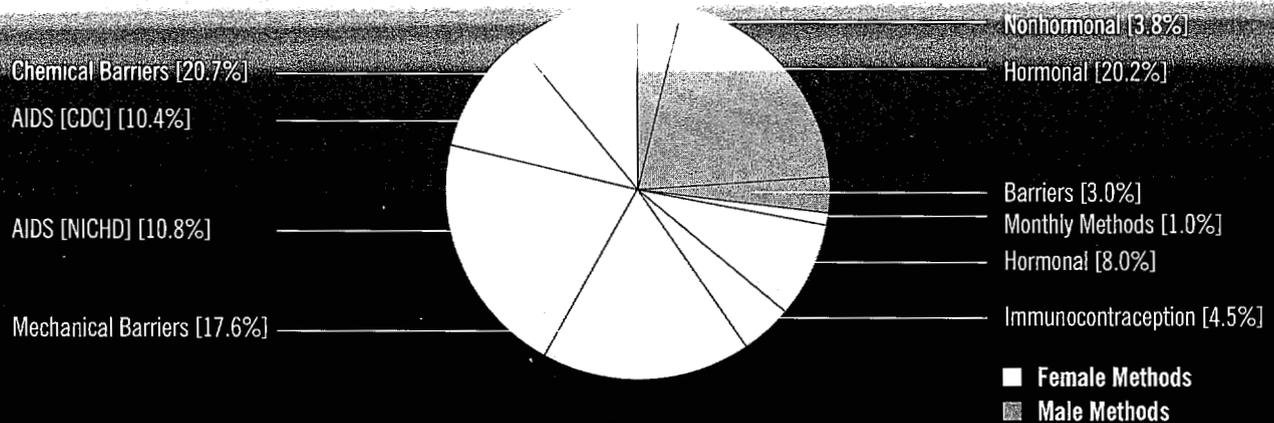
2. Microbicides to prevent the transmission of HIV/AIDS and other STDs

3. Microbicides to prevent the transmission of HIV/AIDS and other STDs

4. Microbicides to prevent the transmission of HIV/AIDS and other STDs

The following sections describe CONRAD-supported projects in these five areas that have been completed recently, are underway, and/or are in the planning stage.

CONRAD PROJECTS Active in 1997 (in percent by dollar allocation)



Contraceptives for Use by Women

MECHANICAL AND CHEMICAL BARRIERS

Because of the need for methods that not only prevent pregnancy but also reduce the transmission of STDs, including HIV, CONRAD continues to give highest priority to barrier contraceptives. Two major challenges must be met in developing new barrier contraceptives to prevent pregnancy and disease:

- Microbial attachment and penetration of the vaginal wall must be prevented. This is a relatively new area of contraceptive research because protecting or coating of the vaginal wall generally is not needed to prevent pregnancy.
- Disruption of the vaginal environment must be minimized. High doses or frequent use of nonoxynol-9, a widely used spermicide, can disturb the vaginal epithelium and upset the vaginal flora. Disruption of the vaginal epithelium is a concern because it may create a portal of entry for STD-causing organisms. Also, vaginal irritation—even if minor—can contribute to reduced acceptability of the product.

CONRAD has completed or is working on several projects involving barrier methods, including:

- two cervical barriers—the Lea® Contraceptive (previously called Lea's Shield) and FemCap™;
- a silicone diaphragm;
- new spermicidal formulations; and
- male condoms.

Several studies on new barriers that prevent STD transmission as well as pregnancy are being carried out in partnership with private sector organizations. Researchers are trying to make these products feasible for use in less developed countries by ensuring that they can be manufactured locally, do not require frequent resupply, and can be disposed of easily.

The current market for all over-the-counter (OTC) products is small (about \$40 million in the United States). However, development of safe and effective vaginal methods that may be used privately by women in anticipation of the need should expand the current, limited market for barrier methods. A study funded by CICCRR will provide additional information on users' perceptions and needs and market estimates for a dual product.

Mechanical Barriers

Lea® Contraceptive. In collaboration with Family Health International (FHI), CONRAD sponsored Phase I, Phase II, and OTC feasibility studies of Lea® Contraceptive. This silicone rubber device covers the cervix and, when used with spermicide, appears to prevent pregnancy at least as well as traditional barriers. Earlier reports showed that the six-month cumulative life table pregnancy rate was 8.7 per 100 women. However, data submitted to FDA from the Phase II trial were deemed insufficient for an approval recommendation. Thus, CONRAD and FHI have designed an expanded Phase III safety, acceptability, and contraception efficacy trial for the device as an OTC product. The trial also will include a colposcopy substudy at two centers. The study will be carried out from 1998 through 2000.

FemCap™. A Phase II/III study of FemCap™, a silicone rubber cervical cap that comes in three sizes, was completed in 1997. FemCap™ has several advantages over existing barrier devices. It has more durable and less sensitizing materials, fewer dislodgments, a lower risk of urinary tract infection (UTI), and size determination based on obstetrical history. Also, its design conforms with the anatomy of the cervix and proximal vagina to provide a snug, comfortable fit, making it difficult to dislodge.

This study evaluated the contraceptive efficacy, safety, acceptability, and fitting characteristics of FemCap™ compared with the standard latex diaphragm. Seven hundred ninety-nine women at risk for pregnancy were enrolled at 10 centers in the United States. An additional cohort of 42 women underwent colposcopy at regular intervals during the study as an additional safety evaluation. FHI was funded by CONRAD to assist with the clinical monitoring and data analysis.

At six months, the cumulative, typical-use rate was 13.5 per 100 women among FemCap™ users, significantly higher than the 7.9 rate among diaphragm users. However, pregnancy rates among both groups were well within the efficacy range for barrier methods (see Table 1). In previous studies, the Prentif Cavity-Rim cervical cap had a six-month pregnancy rate of 12.8 and a diaphragm rate of 11.0. FemCap™ was associated with significantly fewer urinary tract infections. In addition, the majority of volunteers liked the device, said they would use it, and would recommend it to a friend.

Table 1 Safety and Efficacy of FEMCAP™ Comparison Phase II/III Trial with the Diaphragm—Preliminary Results

	FemCap™ (N=355)	Diaphragm (N=403)
Six-Month Cumulative Life Table Pregnancy Rate per 100 Women	13.5	7.9
Cumulative Discontinuation Rates for Reasons other than Pregnancy		
• Inability to insert/remove device	1.1	0.0
• Personal/device-related	4.6	1.5
• Personal/not device-related	5.7	6.3
• Medical/safety reasons	5.5	5.8
• Protocol violation	7.1	5.3
• Lost to follow-up	3.7	4.3
Acceptability		
• Percent liking device "a lot or somewhat"	78.3	82.3
• Percent who would recommend device	89.1	95.7

Obstetrical history alone determined size for the vast majority of women (85 percent) who could be fitted with a FemCap™. This indicates that OTC availability is feasible. However, some FemCap™ users experienced problems with insertion and, in particular, removal. New prototypes of the device that incorporate a removal strap and were available in only two sizes underwent limited clinical testing to assess ease of insertion and removal in a clinic. Most women in that study preferred the strapped version, usually because of easier removal. However, it is unclear what impact going from three sizes to two would have on efficacy.

FemCap™, Inc., is planning to submit a premarket approval (PMA) application of the cervical cap to the FDA in 1998.

Easy Fit Diaphragm. The Easy Fit silicone diaphragm is being developed by investigators at the Program for Appropriate Technology in Health (PATH) in Seattle with CONRAD collaboration. Each design iteration is the result of fittings and input from volunteers and clinicians. The reusable diaphragm is expected to be marketed as a one-size-fits-all device. Overall fit for the latest prototype was excellent. However, minor changes are still planned for the spring assembly, orientation markings, and removal grip. In 1998, clinical testing of the production version will include Phase I postcoital testing at the CONRAD clinical facilities in Norfolk.

Chemical Barriers

Nonoxynol-9. Nonoxynol-9 (N-9) is the most commonly used spermicide in the United States, but questions remain about optimum dose, timing of administration before intercourse, additional application with subsequent acts, and efficacy in perfect use. In addition, vaginal irritation appears to be associated with high doses or frequent use. CONRAD has directed major efforts at improving N-9 and other existing spermicides and developing new ones.

Previous research by CONRAD of a marketed N-9 film (VCF®) determined the concentration of N-9 in the vagina at different intervals up to four hours after insertion in the absence of intercourse. The concentration of N-9 stayed relatively high for about 90 minutes and then fell, though pieces of undissolved film were still present after four hours in some women. A recently completed study correlated N-9 concentration (released from VCF®) with its ability to prevent the passage of progressively motile sperm into midcycle cervical mucus when intercourse followed VCF® insertion. Data analysis for this follow-up study is continuing, but negligible N-9 was measured at all times. The lack of a temporal dependence may reflect insensitivity of the analysis or interference from vaginal and semen constituents. It is clear that improved methodology is necessary to evaluate the duration of action of N-9 spermicides.

Investigators at Duke University have developed a possible approach to assess and improve the delivery of agents that inhibit the migration, function, and/or survival of spermatozoa, and possibly STD pathogens as well, in the cervical mucus. Collaborations are being established to apply this technology during product development. In the past year, new *in vitro* and *in vivo* techniques were developed to assess the spreading of vehicles over the vaginal epithelium and the external cervical os, and the adhesion of vehicles to these surfaces over time. It is hoped that the specific physical properties that most significantly affect the deployment of vehicles in the vagina can be identified and used to predict factors such as the extent of tissue coverage, depth of coating, and maintenance of adhesion. This information will then be used to optimize the contraceptive and prophylactic performance of vaginal formulations *in situ*.

A complementary method for assessing the spreading of active agents and vehicles will be tested this year at the University of Kentucky. Gamma scintigraphy combines radiology imaging with different pharmaceutical dosage forms. Test drugs and vehicles will be radiolabeled, and their distribution and elimination rates in the vaginal cavity will be monitored. When this technique is validated, it may provide an additional tool for the formulation of potential products.

Collaborators at Integra LifeSciences Corporation are exploring a number of new formulations for N-9 to optimize bioavailability while minimizing dose, thereby reducing the likelihood of irritation. The CONRAD/Integra collaboration has resulted in the development and patenting of Q2, a hydrophobe-modified cationic polysaccharide excipient for topical microbicide/spermicide formulations that reduces N-9 irritation in animal models and has high *in vitro* efficacy. A Phase I safety study using preloaded applicators of Q2 plus N-9 plus dextran sulfate will begin in 1998 at CONRAD's clinical facilities in Norfolk. A successful outcome from this study will be followed by Phase I postcoital testing in early 1999. Additional stability and toxicology testing and applicator development are underway.

CICCR continues to collaborate with investigators in the Program for the Topical Prevention of Conception and Disease (TOPCAD) at Rush-Presbyterian-St. Luke's Medical Center in Chicago and a commercial partner in developing a new long-acting, sustained-release spermicide formulation of N-9. A Phase I trial on safety (irritation) after varying doses of N-9 is complete. Preliminary data were adequate to proceed with the design of a Phase I postcoital test. This study will be completed by mid-1999, when, it is hoped, it will proceed to a Phase II efficacy trial. TOPCAD investigators also are collaborating with clinical investigators at CEMICAMP in São Paulo, Brazil, to prepare clinical grade materials and test a new bioadhesive, N-9-containing, low pH formulation for safety and Phase I efficacy on a limited number of women in Brazil.

Sperm-Agglutinating Antibodies. An alternate barrier strategy uses formulations that contain monoclonal antibodies to completely agglutinate sperm, and possibly STD pathogens, in the ejaculate. Investigators at the University of Virginia have identified one such antibody that rapidly immobilizes sperm and prevents sperm-egg binding *in vitro*, and have characterized the cognate sperm antigen. The monoclonal antibodies are currently being formulated and tested in a proprietary liposome vehicle in anticipation of Phase I clinical trials by 1999.

Future Directions

CONRAD maintains a major interest in barrier methods, including chemical barriers. In particular, CONRAD supports clinical studies to define the duration of action for N-9 products and the safety and efficacy of improved formulations of N-9 and other active agents. Several groups, including CONRAD, are trying to standardize colposcopy techniques and analysis to better evaluate vaginal irritation and any associated changes in risk of STD transmission concomitant with spermicide/microbicide use. In addition, work will continue with industrial partners to develop new chemical entities that show both contraceptive and antimicrobial promise, and preclinical screening of candidate products will continue.

Promising leads have been identified with high spermicidal and virucidal activity.

MICROBICIDES FOR THE PREVENTION OF HIV/AIDS AND OTHER STDs

The three major goals of CONRAD's research in this area are:

- development of new products that prevent the transmission of HIV/AIDS and other STDs;
- better definition of how existing and potential contraceptive methods might either reduce or enhance the risk of HIV/AIDS and other STDs; and
- gathering information for more appropriately tailored and responsible family planning services, including improved counseling and products.

Development of New Microbicidal Agents and Formulations

Researchers at the Southern Research Institute in Birmingham, Alabama, and Frederick, Maryland, are using *in vitro* models to evaluate the ability of various compounds to inactivate cell-free and cell-associated HIV or to prevent the attachment of HIV to target cells in the reproductive tract. A significantly expanded testing algorithm is being applied to enhance the characterization of active compounds by looking at activity against virus strains with different types of target cells. Promising leads have been identified among several classes of compounds, including sulfated polysaccharides and other polymers, with high spermicidal and virucidal activity and potentially low levels of tissue irritation. CONRAD will continue to work with investigators in the United States and abroad to refine and improve these compounds.

Another *in vitro* investigation supported by CONRAD has generated immortalized cell lines from human vaginal and cervical epithelia. This year, investigators at Brigham and Women's Hospital in Boston will use these novel cell lines to investigate the release of specific inflammation markers, such as selected cytokines and chemokines, following cell

New information on heterosexual transmission of HIV continues to emanate from the SIV model developed with funding from CONRAD.

exposure to potential new agents with spermicidal and/or microbicidal activity. The objective of these studies is to identify robust markers for toxicity and inflammation that can be used as a new screening assay before *in vivo* irritation testing is undertaken.

CICCR has provided substantial funding to TOPCAD to evaluate and develop a number of promising agents with antifertility and/or anti-STD properties. TOPCAD investigators have identified two highly promising leads, both of which have attracted matching funding from industry as part of CICCR. Preliminary screening of two high molecular weight sulfated polymers (one a polysaccharide) demonstrated *in vitro* anti-HIV, anti-HSV, and antigonococcal activity. They appear to be nonirritating to the vagina, inhibit sperm function *in vitro*, and have very promising antifertility activity *in vivo* in the rabbit model. Owing to their large molecular weights, their systemic absorption from the vagina is unlikely. In addition, the polysaccharide possesses gel-forming properties that may make it a very desirable carrier material for agents other than N-9. Initial formulation work, stability testing, and animal safety studies are underway; Phase I safety studies in women are on track to begin in 1998.

Selection of the appropriate delivery vehicle—gel, cream, suppository, film, or sponge—is often a complex process that requires the integration of chemical properties, *in vitro* testing, manufacturing and economic limitations, and many other factors. In view of this difficulty, CONRAD, in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), sponsored a workshop on vaginal microbicide formulations in November 1997. The published proceedings will be available by summer 1998.

Mechanisms of Sexual HIV Transmission

For several years, investigators at Harvard University have examined the mechanisms and cofactors of HIV secretion. These studies initially focused on the male reproductive tract, but as AIDS prevalence in women increased, the investigators expanded their efforts to include the female reproductive tract as well.

In the male reproductive tract, investigators identified cells in semen that carry HIV, the tissues from which infected cells and free virus are secreted, and their relationship to disease stage, therapy, and immune function. Completed studies found that:

- vasectomy reduces but does not prevent HIV in the ejaculate;
- HIV in semen is greatly reduced by zidovudine (AZT) treatment;
- sperm do not carry HIV; and
- survival of free and cell-associated HIV is highly pH dependent.

More recently, these researchers have used surgical and autopsy tissues from HIV-positive women to study the localization and secretion of HIV in the female reproductive tract. Data have been correlated with disease stage; pathological condition; therapy; coinfections such as cytomegalovirus, human papillomavirus, and Epstein-Barr virus; and other factors, such as menstrual stage and endometriosis.

As a general rule for men and women, HIV secretion in the reproductive tract is associated with local inflammatory responses of various etiologies. Work is underway to identify the inflammatory factors in lower genital tract secretions associated with HIV-1 shedding.

During the past year, this work has taken a much more clinical approach. Quantitative methods that measure the release of cytokines, chemokines, and other factors are being used to assess inflammation in reproductive tract tissues of women who have used an N-9-containing spermicide with and without condom-protected intercourse. These data will shed light on how these factors are related to HIV transmission and how they are influenced—positively or negatively—by contraceptive methods and sexual practices. It is anticipated that the same techniques can then be used to investigate the influence of other contraceptive methods such as oral contraceptives and injectable steroids.

Development of Animal Models for HIV Transmission

New information on the heterosexual transmission of HIV continues to emanate from the rhesus monkey and simian immunodeficiency virus (SIV) model developed at the California Regional Primate Research Center in Davis with funding from CONRAD and the National Institute of Child Health and Human Development (NICHD). In this model, SIV, the monkey correlate of HIV, is transmitted by genital exposure among male and female monkeys. Infection results in a disease with a pathology similar to that of AIDS. Previous research characterized the relevant biology in this model

and demonstrated the partially protective effects of N-9-containing foam and gel contraceptives against highly infectious inocula of SIV. Additional studies are planned for this year to assess the ability of several potential new products to prevent the establishment of vaginal SIV infection in this model. This evaluation could be a significant milestone in the development of new products to protect against the sexual transmission of HIV to women.

A rigorous regimen of exposure to an N-9-containing vaginal spermicide (twice a day for 28 days) did not increase the likelihood of SIV infection following vaginal inoculation, the Davis investigators found in a preliminary study. They used video colposcopy and histological analysis of vaginal biopsies to produce extensive characterizations of the effects of this and other treatment regimens, including controls and a group treated for 42 days. Following 42 days of twice-a-day administration, significant lesions were found that persisted for at least seven days after the last N-9 exposure. NIAID is funding the continuation of these studies.

At the Aaron Diamond AIDS Research Center in New York, investigators found that a significantly higher percentage of progesterone-implanted monkeys became infected following vaginal inoculations of SIV, compared with placebo-implanted monkeys. Histological evaluations indicated that this enhanced transmission might be attributable to the progesterone-induced thinning of the vaginal epithelium, though it is unclear what relevance this finding has to the use of progestin-containing contraceptives by women. To answer that question, the CONRAD Clinical Research Unit in Norfolk is comparing vaginal epithelial thickness in women before and during the use of Depo-Provera (depomedroxyprogesterone) and will attempt to correlate these results with the changes observed in the nonhuman primate. A related epidemiological study funded by CDC is underway in Thailand.

Activity of Existing and New Agents against Other STDs

Using the pigtail macaque model of sexually transmitted chlamydial infection, researchers at the University of Washington studied the effects of N-9 and benzalkonium chloride on infection and vaginal flora. A 4 percent N-9 formulation administered in a single vaginal application inhibited infection with only minimal temporary changes in vaginal flora and mild irritation to the vaginal and cervical mucosa. By comparison, a 1.2 percent benzalkonium chloride formulation also protected against infection, but induced more marked disruptions of vaginal flora and irritation. This model will be used to evaluate the *in vivo* antichlamydial activity of other lead spermicidal and/or microbicidal candidates under CONRAD consideration.

Other CONRAD researchers, including those in the TOPCAD, are conducting *in vitro* investigations to identify agents that are effective against a variety of microbes—such as chlamydia, herpes simplex virus, gonococci, hemophilus, trichomonas, and candida—and have minimal impact on normal lactobacilli.

The importance of using condoms for STD protection with hormonal methods has become increasingly apparent.

Epidemiological Studies

With funding provided by CDC, investigators in Chiang Mai, Thailand, are continuing their efforts to identify factors associated with the sexual transmission of HIV, including contraceptive practices, types and frequency of sexual contact, STDs, and other possible cofactors. The objective of this epidemiological study is to identify factors associated with infectiousness from those associated with susceptibility. Researchers are mapping the dynamics of heterosexual HIV transmission cross-sectionally by tracking the serostatus of the female sexual partner(s) of seropositive index males. Nearly half of the enrolled women are HIV-positive. Less than 2 percent of the male index cases report always using a condom with their regular partner, although 98 percent had a history of sex with a female prostitute. HIV-infected male blood donors are also being studied for hepatitis infections. Preliminary results suggest that hepatitis B-antigen-positive blood donors were more likely to have transmitted HIV to their female partners.

Semen samples are being collected to quantitatively measure HIV load by polymerase chain reaction (PCR) techniques and will be related to seroconversion of the female partners. The parameters of women who remain seronegative despite exposure to seropositive men are being compared with those of women who seroconvert upon exposure. The rate of male-to-female HIV transmission among 184 couples appears to be positively associated with plasma viral load (HIV-1mRNA) in this population. Preliminary findings from 30 semen donors indicate that HIV-1mRNA levels correlate relatively well between blood plasma and seminal plasma mRNA.

In response to recent indications from a monkey model that progesterone-induced vaginal atrophy increases the risk of SIV transmission, plans for a large epidemiologic study on Depo-Provera use and HIV transmission in women of reproductive age in northern Thailand are underway. In preparation for the large study, an ongoing preliminary investigation will first determine the feasibility of conducting the large multicentered retrospective cohort study.

In light of new evidence concerning the relationship between the use of progestins and vaginal atrophy, the importance of using condoms with hormonal methods such as Norplant® and Depo-Provera has become increasingly apparent. Consistent use of condoms with Depo-Provera is particularly important, as underscored by the sharp rise in the incidence of HIV infection and other STDs among reproductive-age women. Investigators at Baylor College of Medicine in Houston recently launched a study to identify psychosocial factors that best predict condom use among women who use injectable contraceptives.

Market Analysis of Microbicide Interest

CICCR provided funding for the Alan Guttmacher Institute (AGI) to evaluate the interest of women in using microbicides.

By surveying a nationwide sample of American women, AGI will assess:

- the perceived need for microbicides;
- levels of interest in using them and possible barriers to use;
- preferences regarding various method characteristics; and
- variations among subgroups of the population.

Information from this study will help a variety of stakeholders—including health care providers, advocacy and consumer groups, financiers of method development, and companies interested in manufacturing and marketing such methods—gauge the interest and characteristics of potential users and adapt their methods and marketing strategies accordingly. Study data will be combined with current industry data on spermicide marketing to construct a resource volume for use by private companies in deciding whether and how to become involved with microbicide development and marketing. Additional funding for this project has been provided by The Rockefeller Foundation and The Andrew W. Mellon Foundation.

Future Directions

Future studies regarding the heterosexual transmission of HIV are likely to address the effect of steroids on epithelial structure and the role of additional contraceptive methods, specific STDs, and other phenomena as cofactors for transmission of HIV to or from infected individuals.

SYSTEMIC HORMONAL METHODS

CONRAD continues to investigate the ability of long-acting hormonal delivery systems to prevent pregnancy. Earlier activities in this area have focused on methods that are suitable for lactating women and/or that use natural rather than synthetic steroids, such as progesterone-releasing vaginal rings and microspheres. More recent efforts include the development of a progestin-releasing intrauterine device (IUD) that would deliver hormone locally and a progestin-releasing single implant.

Progesterone Vaginal Rings

Research continues on progesterone rings at CONRAD's collaborating research center, ICMER, in Santiago, Chile. Recently, a pharmacokinetic study of the latest ring manufactured by Silesia Laboratories in Santiago indicated that the rings would be effective for up to four months. A Phase II trial for efficacy will begin in 1998.

Levonorgestrel-Releasing Devices

In order to provide a simpler, low-cost alternative to progestin implant systems on the market, development of a single-rod levonorgestrel implant is underway in collaboration with FEI Technologies, Inc. In 1998, a Phase I pharmacokinetic and safety study will begin in Norfolk, comparing the one-rod implant to the six-capsule Norplant® system.

Additional ongoing collaborations with FEI are underway to develop a levonorgestrel-releasing IUD and possibly a vaginal ring. The IUD project is still in the design stage; the intent is to produce an IUD that may lower pelvic inflammatory disease risks. Previous multicenter studies by the World Health Organization (WHO) of a vaginal ring that released 20 mg of levonorgestrel were moderately encouraging, but pregnancy rates, vaginal irritation, and expulsion rates were suboptimal. A new vaginal ring is being developed with greater flexibility and increased levonorgestrel release.

Scientific data about the way in which progestogenic forms of hormonal contraception exert their contraceptive action on cervical mucus are inadequate, even though mucus thickening is known to be a key mode of its action. CICCR-funded investigators at the University of Hull, England, plan to sample cervical mucus from women in a number of clinical situations, including Norplant® use, to establish laboratory parameters that can be equated with lack of fertility.

Future Directions

CONRAD believes that their very high efficacy provides a firm basis upon which to continue to pursue improved hormonal methods for women. However, the previously reported data on enhanced SIV transmission under the influence of progesterone give reason for caution. CONRAD will continually pay appropriate attention to new risk assessments. The results of the study on the impact of Depo-Provera on the vaginal epithelium in women are not yet available. On the other hand, since the available cumulative epidemiologic evidence on HIV transmission does not indicate increased risks from progestin contraceptive use, CONRAD will continue to develop improved delivery systems.

U.S. & INDIA

U.S. AND INDIA COLLABORATE ON CONTRACEPTIVE AND REPRODUCTIVE HEALTH RESEARCH

Building on previous collaborative research efforts, the governments of the United States and India agreed in 1997 to a new five-year initiative that will result in expanded contraceptive options and improved reproductive health.

The initiative recognizes the importance of voluntary family planning and improving reproductive health for individual, family, and societal well-being.

The U.S. partners are the Department of Health and Human Services and the Agency for International Development. CONRAD is charged with implementing the USAID part of the program, which will include organizing meetings and sponsoring collaborative research.

The Indian partners are the Department of Biotechnology, the Ministry of Health and Family Welfare, the Indian Council of Medical Research, and the Council of Scientific and Industrial Research.

Drawing on the expertise of U.S. and Indian scientists and institutions, the initiative will initially pursue work in eight scientific areas. These areas complement other ongoing CONRAD activities.

Male Methods and Male Involvement

Researchers will investigate oral and injectible hormonal approaches, condoms made from latex and other materials, and ways to increase male participation in family planning.

Long-Acting Contraceptives for Women

Research on long-acting preparations for women will include progestin only and monthly injectibles. In addition, researchers will pursue acceptability and behavioral studies to improve use and continuation rates.

Conception and STD Prevention

Recognizing that the use of barrier methods can provide protection against unplanned pregnancy and STDs when used correctly and consistently, researchers will work on developing new, effective, acceptable, affordable, and safe methods, including male and female

condoms and spermicides and microbicides, and improving use of existing and new methods.

Emergency Contraception

The availability of methods that prevent pregnancy after unprotected intercourse is vital to women's reproductive health. This initiative will promote existing and new emergency contraceptive pills and investigate ways to increase their availability and use.

Social and Behavior Research

In addition to acceptability research conducted as part of contraceptive trials, collaborative efforts will focus on social and behavioral research to better understand the factors affecting contraception and reproductive health.

Epidemiology

Researchers will pursue epidemiological studies to better understand the relationship between contraceptive methods and health-related issues, including STD and HIV protection and susceptibility.

Immunocontraception

The development of immunocontraceptives (contraceptive vaccines) faces numerous scientific and social hurdles. New research under this initiative will follow several potential leads to the clinical evaluation stage, conduct clinical trials of products that have been approved by regulatory bodies for research in humans, and examine the related social and health issues.

Basic, Applied, and Clinical Research in Reproductive Health

Although contraception, family planning, and the prevention of STDs and HIV are the priority areas of research under the initiative, work also will cover basic, applied, and clinical research on male and female reproduction, including reproductive tract disorders.

In addition to these areas of research, new topics in contraception and reproductive health can be considered for support under the collaborative agreement.

SYSTEMIC NONHORMONAL METHODS

Immunocontraceptives

Development of a new contraceptive method for women without the side effects typical of current hormonal methods, but with sustained duration of action, low manufacturing costs, and simple administration, holds obvious attractions. For this reason, CONRAD has included immunocontraception as a priority research area. However, CONRAD recognizes that significant hurdles must be overcome to achieve an immunologically based method, including:

- lack of perfect animal models;
- lack of optimal adjuvants and delivery systems;
- variability in immune responses;
- lack of protection against STDs; and
- reversibility.

Therefore, these concerns, along with the lack of highly promising leads from animal trials and the limited funding available to CONRAD for long-term leads, have led to more cautious support in this area.

Sperm Antigens. CONRAD has pursued a number of leads based on sperm antigens. Monoclonal antibodies generated against human sperm have been useful in defining candidates and producing purified sperm antigens. Unfortunately, antifertility trials targeting some sperm antigens in small animal models have tended to demonstrate moderate to high efficacy that has not always been replicated in the more expensive and time-consuming primate trials. Primate fertility trials of other sperm antigen leads, such as the sperm isomer of lactate dehydrogenase, LDH-C4, have demonstrated only moderate antifertility effects.

In a twinning-funded collaboration, investigators at the University of Virginia and the National Institute of Immunology in New Delhi, India, are attempting to isolate and characterize gene-encoding sperm surface antigens by screening human testis gene libraries. At least one antigen appears promising and is being further characterized as an immunocontraceptive for testing in a primate model.

Zona Pellucida Antigens. The zona pellucida (ZP), an extracellular matrix surrounding mammalian oocytes, mediates the initial recognition and binding of sperm to oocytes and subsequent activation steps during fertilization in a species-specific manner. This critical role in reproduction has made ZP proteins potential candidate antigens for immunocontraception. Significant infertility can result from active immunization with ZP purified from native material, though often in concert with ovarian pathology. In order to eliminate adverse effects on the ovary, ongoing CONRAD projects focus on identifying epitopes that do not induce ovarian pathology and on testing the immunogenicity and contraceptive potential of recombinant ZP peptides.

Investigators at the National Institute of Immunology in New Delhi have immunized bonnet monkeys with recombinant bonnet monkey ZP subunits and synthetic peptides of ZP that correlate with peptides that are important for sperm-zona binding in other species. Although fertility was reduced in several immunized groups, the number of females per mating group has been small, precluding meaningful interpretation. Ovarian disturbances have also been noted in the group of monkeys that were immunized with one recombinant ZP component (ZP-3), although they were reversible. As a result, investigators have begun primate immunogenicity and antifertility studies using different carrier conjugates and alternative ZP antigens (e.g., ZP-1), which may induce less ovarian disruption.

Investigators at Baylor College of Medicine have generated high-antibody titers against a recombinant rabbit homolog of human ZP in primates, although significant antifertility effects were not observed. Previous immunizations have generated antibodies that block primate sperm-zona binding *in vitro*, induce the acrosome reaction in primate sperm, react with primate zona, and do not induce ovarian pathology. Epitope mapping studies were then undertaken to identify the most important epitopes for human sperm-zona binding. Two major overlapping human ZP peptide epitopes have been identified. These antibodies will be used to obtain affinity-purified antibodies to evaluate their antifertility potential by *in vitro* sperm-zona binding inhibition experiments. Part of this continued effort is supported by CICCR twinning funds.

Future Directions

CONRAD will continue to evaluate viable immunocontraceptive leads as they arise, support refinement of the most promising current leads using critical peer review, and, working with outside consultants, assist investigators with (new) antigens to optimize formulations and delivery systems that would be appropriate to test in nonhuman primates. In addition, minimal criteria have been established for CONRAD's involvement with a candidate antigen. Ideally, the native antigen should:

- be well-characterized (i.e., its role in fertilization established and amino acid sequence known);
- be tissue-specific;
- have demonstrated *in vivo* and *in vitro* efficacy; and
- be producible using recombinant DNA technology.

The strength of these data will determine support for reassessing *in vitro* and *in vivo* efficacy using the recombinant protein and for optimizing the immunogen. Potential links to prevention of STDs would be viewed favorably. In spite of the current lack of highly promising leads, CONRAD believes that immunocontraceptives are an innovative approach and should be pursued as long as the goal of developing effective, inexpensive, and easy-to-provide methods of fertility regulation can be met.

Contraceptives for Use by Men

With essentially only one reversible male method of contraception—the condom—on the market, the development of new male contraceptives continues to be a high priority for CONRAD. Although currently available latex condoms are relatively effective in preventing sexual transmission of infectious disease agents, they are unacceptable for many men and are much less effective in preventing pregnancy than methods readily available to women.

CONRAD has tended to give priority to developing systemic methods focused on suppression of spermatogenesis. Suppression is achieved by exogenous administration of hormones that inhibit the production and release of endogenous male hormones critical for spermatogenesis. Most of the ongoing clinical studies involve suboptimal prototype drug formulations and are being assessed in small numbers of men. CONRAD continues to investigate nonhormonal methods as well, including nonlatex condoms, immunocontraceptives, and post-testicular agents.

MECHANICAL BARRIERS

Tactylon®

The development of improved male condom materials that resist breakage and deterioration when stored under adverse conditions and of more comfortable condom designs could increase condom efficacy, acceptability, and, ultimately, use for contraception and STD prevention. Tactylon®, a synthetic thermoplastic elastomer developed by Tactyl Technologies, Inc. (now called Sensicon Corporation), does not cause allergic reactions in persons sensitive to latex and is more resistant to degradation than latex. In an effort to overcome some of the disadvantages associated with latex material, three styles of condoms from Tactylon® were produced and studied clinically for breakage and slippage in comparison with a latex condom control.

Complete slippage rates were equivalent among the Tactylon® condoms and the latex condom. However, the Tactylon® condoms broke slightly more often during use. Acceptability appeared to be best for two of the Tactylon® condoms. All three Tactylon® condoms were better than the latex condom in terms of irritation. The company used the CONRAD results as part of its premarket notification clearance (510[k]) application, which was approved for one style in 1997 and a second style in early 1998.

Future Directions

CONRAD's continued involvement in male condom development will probably be limited, since a number of organizations are actively engaged in this area. However, a new latex condom that offers greater acceptability may be evaluated in the context of the U.S.-India collaborative research program.

SYSTEMIC HORMONAL METHODS

Gonadotropin-releasing hormone (GnRH) is a hypothalamic peptide that stimulates pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In males, FSH stimulates and maintains spermatogenesis and LH acts on testicular cells to stimulate testosterone production, which is critical for spermatogenesis. Contraceptive approaches tested by CONRAD and other organizations have largely centered on feedback inhibition of FSH and LH release by administration of GnRH antagonists or exogenous androgens alone or in combination with other sex steroid hormones. These regimens lead to suppression of testosterone in the short term and inhibition of spermatogenesis in the long term. When exogenous administration is terminated, sperm production returns to normal.

Androgen/Progestin Combinations

CONRAD has supported several studies designed to determine whether the addition of a progestin increases the proportion of men who achieve azoospermia and permits the use of less testosterone (thus inducing fewer side effects such as acne, weight gain, or adverse reductions in serum lipids, especially HDL-cholesterol). The latest data from the University of Washington researchers confirm that men who receive daily doses of the synthetic progestin levonorgestrel (LNG) plus weekly testosterone enanthate (TE) suppress sooner and to a greater extent than men who receive only TE (see Table 2). Effects on serum lipids appear to be dose-related. Men who received the lowest-tested LNG doses experienced less of a reduction in serum lipids than men who received the highest dose. Further investigation of this promising approach is warranted. Studies recently were initiated to identify the lowest dose of LNG in combination with TE that will suppress spermatogenesis adequately without adversely affecting serum lipids.

In women, oral contraceptive pills containing third-generation synthetic progestins, such as desogestrel, seem to have less of an adverse effect on lipids than oral contraceptives containing other progestins, such as LNG. Investigators at the

Table 2 Androgen plus Progestin Combination for Systemic Male Contraception

Hormone Regimen	Proportion Achieving Azoospermia or Oligospermia (<3 million/ml)	Time to Azoo- or Oligospermia (weeks)	HDL-Cholesterol (percent change between control and treatment cycle)
TE alone (100 mg weekly)	61%	14.4	- 4%
TE weekly +500 µg LNG, daily	94	8.9	-22
TE weekly +250 µg LNG, daily	89	7.7	-20
TE weekly +125 µg LNG, daily	94	9.3	-13
TE weekly +63 µg LNG, daily	To be determined		To be determined
TE weekly +31 µg LNG, daily	To be determined		To be determined

University of Washington and the University of Manchester led a multicenter study that sought to verify whether lipids are adversely affected in men when desogestrel is used as the progestin in combination with TE for suppression of spermatogenesis. That project found that sperm suppression to azoospermia or severe oligospermia was achieved in almost all men; nevertheless, HDL-cholesterol was reduced significantly. A follow-up study using oral desogestrel at lower doses and in combination with a transdermal testosterone patch is underway at Manchester.

Preliminary results indicate that progestin/androgen combinations are more effective and safer than androgen alone. However, daily administration of a progestin/androgen combination is not an acceptable male contraceptive formulation because it may lead to pregnancy if the male does not use it consistently. Because of this shortcoming, long-acting testosterone and progestin esters developed by WHO and NIH researchers are more attractive. Previous preliminary studies using testosterone bucylate in men and LNG butanoate in women showed promising results. However, additional development of these particular formulations needs to be completed before entering into additional clinical studies in men. Other long-acting formulations are being investigated in the interim.

Investigators at Harbor-UCLA Medical Center have initiated a new study to compare the efficacy of a long-acting LNG formulation, Norplant® II (developed by The Population Council), in combination with a new transdermal androgen (developed by Alza Corp.) versus transdermal androgen alone in suppression of spermatogenesis in normal man. Results from this trial will be compared to findings from ongoing studies at the University of Turku, Finland, in which the combination of Norplant® II and another transdermal androgen cream is being tested.

Androgens in Combination with Other Suppressive Agents

CICCR is supporting three studies of androgen formulations in combination with either estrogen or an anti-androgen. Two of these studies are clinical trials that are benefiting from significant industrial involvement. The safety and efficacy of low-dose estradiol supplementation for depot testosterone-induced suppression of human spermatogenesis is under investigation at the Royal Prince Alfred Hospital, Camperdown, Australia. Investigators at the University of Bologna, Italy, will carry out a trial of the combination of the anti-androgen, cyproterone acetate (CPA), and testosterone undecanoate in 1998.

Primate studies have indicated that estradiol supplementation can significantly enhance the suppression achieved by depot testosterone. However, this observation has not been tested in men, mainly due to the absence of adequate depot androgen formulations as well as low-dose depot formulations of estradiol. Preliminary results obtained by the Australian investigators indicate that the estradiol dosage is too small to produce any measurable change in serum estradiol levels. As a result, the protocol has been modified to increase the estradiol dosage and continue the study.

Unlike testosterone, the androgen 7 α -methyl-19-nortestosterone (MENT) does not metabolize into a more potent androgen and, consequently, is not expected to adversely affect prostate growth. In animal models, this drug is more potent for gonadotropin suppression than testosterone. This compound, under development by The Population Council, is a highly promising lead for a new male contraceptive. CICCR twinning funding is being used by The Population Council and the Indian Institute of Science to test MENT alone and in combination with estradiol in a monkey model.

In pilot trials in Italy, investigators have shown that the combination of cyproterone acetate (CPA) and TE produced complete azoospermia in all men treated. This is a significant result, considering that azoospermia is not uniformly achieved in men who receive a combination of levonorgestrel and TE. Subsequent studies, however, indicated that this result was dependent on the CPA dosage given. An expanded CPA dose-finding Phase I study is set to begin in which men will receive CPA in combination with a new formulation of testosterone undecanoate (TU) that appears to provide stable blood levels of testosterone for at least 60 days.

GnRH Analogs

GnRH antagonists, which inhibit pituitary FSH and LH secretion in both women and men, may provide a useful approach for developing a practical male contraceptive. However, they have several drawbacks. An androgen, such as testosterone, must also be administered to maintain libido and potency. In addition, most antagonists and agonists are relatively expensive synthetic peptides and require frequent (if not daily) administration; some of them can induce local skin irritation.

Investigators at the University of Washington and Harbor-UCLA Medical Center have nearly completed studies on ways to eliminate the need for long-term, daily administration of the antagonist Nal-Glu (Lupron®). Investigators

found that androgens alone (TE, in this case) can maintain azoospermia or severe oligospermia induced with a priming dose of Nal-Glu plus TE for at least 20 weeks. While this result is encouraging and this regimen could significantly reduce the cost of a GnRH antagonist-based male hormonal method, the ultimate cost and availability of an antagonist leave this approach in doubt.

New GnRH antagonists are being developed continually by private companies and other public sector organizations with the hope of increasing potency (thus reducing needed dosage) and decreasing side effects and costs. It is not known whether a potent nonpeptide antagonist will be identified that does not need to be administered daily. However, if any of the new antagonists appear highly promising in preclinical or clinical testing, CONRAD will remain interested in participating in additional clinical trials.

GnRH peptide agonists are cheaper to produce than the antagonists, but earlier studies that used GnRH agonists failed to significantly suppress spermatogenesis. Because those trials may have used an insufficient dosage, investigators at the University of Washington and Harbor-UCLA Medical Center conducted a study in normal men using higher doses of the agonist D-trypt⁶-Net GnRH to confirm or reject this assumption. Unexpectedly, very few men who received daily injections of the agonist plus TE achieved either azoospermia or significant oligospermia. As a result, this approach will not be pursued further.

Future Directions

CONRAD expects to continue research collaborations on long-acting androgen formulations for male contraception. These products, some of which are already marketed for treatment of hormone deficiency and other medical indications, include 90-day testosterone ester injectables, six- to 12-month implants, and daily transdermal patches. It is hoped that the formulation issues for testosterone bucyclate, a promising ester, will be resolved soon so that CONRAD can continue collaborations with WHO and NIH to resume clinical testing in men by 1999.

Although the most recent results with GnRH antagonists show promise for identifying more acceptable regimens, CONRAD's continued support of GnRH analog-based trials may decline considerably unless analogs can be found that have significantly fewer negative characteristics (i.e., local reactions, high cost, and poor formulations).

CONRAD expects to continue research on long-acting androgen formulations for male contraception.

SYSTEMIC NONHORMONAL METHODS

Testicular and Post-Testicular Agents

Many compounds taken systemically impair fertility, some without significantly reducing spermatogenesis. However, almost all of these agents induce other undesirable side effects or exert varying degrees of toxicity. Although completely novel compounds will require significant preclinical toxicology testing, CONRAD has chosen a few promising leads for mechanism-of-action studies, toxicity tests, and fertility trials. For example, participants in the International Organization for Chemical Sciences in Development have assessed imidazoles and related compounds with putative post-testicular antifertility effects and that could be administered orally. However, most recent studies of the selected compounds found no impairment of fertility in male animal models.

Refined plant extracts used in Chinese medicine have been shown to produce antifertility effects in male mice and men in doses that do not seem to invoke significant toxicity or side effects. One such extract, highly purified triptolide from *Trypterygium wilfordii*, may prove suitable as a male contraceptive if adequate safety and efficacy can be established. Using CICCR twinning funding, investigators at the Wisconsin Regional Primate Center and Mahidol University in Bangkok, Thailand, are collaborating to determine the efficacy and mechanism of action of triptolide in inhibiting fertility in a monkey model.

Several relatively basic projects that focus on systemic male contraceptive development are underway using CICCR feasibility funding. In the first of these studies, investigators at The Population Council are developing analogs of lonidamine (an anticancer drug with antispermatogenic activity) to induce release of premature germ cells in the testis. Preliminary results have been very encouraging, and a for-profit organization has agreed to become a research partner and provide matching funding. Selective inhibition of testis- or sperm-specific isoenzymes may lead to safe and reversible inhibition of sperm function. Investigators at the University of Mississippi are trying to establish whether inhibitors of sperm lactate dehydrogenase (LDH-C₄) may be one such isoenzyme in an animal model, and investigators at Millersville University in Pennsylvania are characterizing a testis-specific isoenzyme of dihydrofolate reductase in hopes of identifying a highly selective inhibitor.

Immunocontraceptives

Follicle-Stimulating Hormone (FSH). Encouraged by results from investigators in India and Germany that demonstrate reversible inhibition of spermatogenesis and subsequent infertility following immunization of nonhuman primates with FSH, CONRAD has supported development of an FSH-based immunocontraceptive. The work is being reviewed continuously, however, since its actual feasibility has not been established.

To provide a reliable and reproducible source of FSH for clinical trials, investigators at the Indian Institute of Sciences (IISc) have produced recombinant FSH in a yeast expression system, albeit at relatively low expression levels. The subunits

are capable of appropriate annealing and bind to FSH receptors to elicit biological responses. In addition, a CICCR twinning project between The Population Council and IISc investigators will pursue the premise that inhibition of FSH action at two levels would provide more effective fertility regulation. The goals of this study are to identify the receptor domains involved in FSH binding and signal transduction and to produce antibodies that can then be used to probe FSH interaction with its receptor. Cell constructs have been generated that produce recombinant FSH receptor domains capable of binding FSH. This is a powerful strategy for understanding how FSH binds to the receptor and elicits its action. However, discouraging results from a recent Phase I clinical trial of men in India and the finding that FSH gene-deleted mice are still fertile strongly suggest that immunocontraceptives based solely on FSH or its receptor will not work.

Epididymal Antigens. Sperm passing through the epididymis mature and acquire fertilizing capacity, suggesting post-testicular modification of the sperm by epididymal proteins. A major challenge is identifying proteins expressed only in the epididymis that might serve as immunocontraceptive targets. Two CICCR-supported projects are examining this objective; one has garnered additional commercial commitments.

Antibodies to recombinant secretory epididymal proteins (initially identified by an industrial partner's ability to screen thousands of gene fragments for tissue specificity) are being characterized for inhibition of sperm-zona binding. Successful *in vitro* results will be followed by immunogenicity and antifertility testing in monkeys at the University of North Carolina at Chapel Hill and in baboons at the Institute for Primate Research in Nairobi, Kenya. Twinning collaborators at the University of California at Davis and at the Instituto de Biología y Medicina Experimental in Buenos Aires, Argentina, have focused on the human homolog of a specific epididymal protein that is important for fertilization in rodents. Immunocontraceptive studies in a primate model are planned at Davis. In both projects, researchers ultimately would have to determine whether an autoimmune response occurs and, if so, whether it can lead to irreversible infertility.

Future Directions

Achieving a nonhormonal systemic method for men will admittedly be more difficult than a hormonally based method. This is somewhat reflected in the fact that these ongoing projects are at relatively earlier stages than those in CONRAD's other priority areas. Nevertheless, CONRAD will continue to pursue new leads primarily in two directions: 1) epididymal antigens that are incorporated onto the sperm surface and are important for fertilizing capacity, and 2) orally active compounds that inhibit sperm function without inducing harmful side effects. Continued interest in testis-specific antigens found on sperm will depend on the demonstration of the lack of any irreversible effects, particularly autoimmune orchitis.

[THE FUTURE]

CONRAD's activities will remain concentrated on progressing from preclinical studies to Phase I and Phase II clinical evaluations. Because it is essential to continue to develop totally new approaches to fertility regulation—and buttressed by support from private foundations—CONRAD also will investigate the feasibility of new leads for male and female methods. In addition, CONRAD researchers will evaluate the potential acceptability of new methods earlier in the development process.

The most significant endeavors planned for the coming years are:

- clinical studies on spermicides and microbicides;
- Phase II clinical trials of steroidal contraceptives for men;
- improved methodology for evaluation of the safety of vaginal devices and drugs;
- pharmacokinetic and efficacy studies of a single levonorgestrel implant in women; and
- development of new intrauterine and vaginal delivery systems for steroids.

SELECTED BIBLIOGRAPHY

Barrier Methods for Women

Castle PE, Whaley KJ, Hoen TE, Moench TR, Cone RA (1997). Contraceptive effect of sperm-agglutinating monoclonal antibodies in rabbits. *BOR*, 56:153-59.

Dunmire EN, Katz DF (1997). Measurement and modulation on nonoxynol-9 diffusion and bioactivity against spermatozoa in human cervical mucus. *Contraception*, 55:115-22.

Dunmire EN, Katz DF (1997). Alteration of human sperm kinematics in cervical mucus due to nonoxynol-9. *Contraception*, 55:209-17.

Mauck C, Glover LH, Miller E, Allen S, Archer DE, Blumenthal P, Rosensweig BA, Dominik R, Sturgen K, Copper J, Fingerhut F, Peacock L, Gabelnick H (1996). Lea's Shield®: A study of the safety and efficacy of a new vaginal barrier contraceptive used with and without spermicide. *Contraception*, 46:329-36.

Mauck CK, Baker JM, Barr SP, Abercrombie TJ, Archer DF (1997). A phase I comparative study of contraceptive vaginal films containing benzalkonium chloride and nonoxynol-9: Postcoital testing and colposcopy. *Contraception* 56:89-96.

Mauck CK, Baker JM, Barr SP, Johanson WM, Archer DF (1997). A phase I comparative study of three contraceptive vaginal films containing nonoxynol-9: Postcoital testing and colposcopy. *Contraception* 56:97-102.

Mauck CK, Baker JM, Barr SP, Johanson WM, Archer DF (1997). A phase I study of FemCap™ used with and without spermicide: Postcoital testing. *Contraception* 56:111-15.

Systemic Hormonal Methods for Women

Croxatto HB, Massai MR, Salvatierra AM, Fuentealba B, Croxatto ND, Laahteenmaki P (1996). Effects of a sequential regimen of mifepristone-medroxyprogesterone acetate on ovarian function, endometrial status and hormone parameters. *Contraception*, 54:79-86.

Systemic Nonhormonal Methods for Women (Immunocontraception)

Afzalpurkar A, Gupta SK (1997). Identification of epitopes of monoclonal antibodies to porcine zona pellucida 3β glycoprotein, a homologue of the mouse/human sperm receptor. *Amer J Reprod Immunol*, 38: 26-32.

Afzalpurkar A, Sacco AG, Yurewicz EC, Gupta SK (1997). Induction of native protein reactive antibodies by immunization with peptides containing linear B-cell epitopes defined by anti-porcine ZP3β monoclonal antibodies. *J Reprod Immunol*, 33:113-25.

Kaul R, Afzalpurkar A, Gupta SK (1997). Expression of bonnet monkey (*Macaca radiata*) zona pellucida-3 (ZP3) in a prokaryotic system and its immunogenicity. *Molec Reprod Dev*, 47:140-47.

O'Hern PA, Liang Z-G, Bambra CS, Goldberg E (1997). Colinear synthesis of an antigen-specific B-cell epitope with a "promiscuous" tetanus toxin T-cell epitope: A synthetic peptide immunocontraceptive. *Vaccine*, 16:1761-66.

Prasad SV, Wilkins B, Skinner SM, Dunbar B (1996). Evaluating zona pellucida structure and function using antibodies to rabbit 55kDa ZP protein expressed in baculovirus expression system. *Molec Reprod Dev*, 43:519-29.

Zhang X, Lou Y, Koopman M, Doggert T, Tung KSK, Curtiss R (1997). Antibody responses and infertility in mice following oral immunization with attenuated salmonella typhimurium expressing recombinant murine ZP3. *Biology Reprod*, 56:33-41.

HIV/AIDS and Other STDs

Duerr A, Warren D, Smith D, Nagachinta T, Marx PA (1997). Contraceptives and HIV. *Nature Medicine*, 3:124

Marx PA, Spira AI, Gettie A, Dailey PJ, Veazey RS, Lackner AA, Mahoney CJ, Miller CJ, Claypool LE, Ho DD, Alexander NJ (1996). Progesterone implants enhance SIV vaginal transmission and early virus load. *Nature Medicine*, 2: 1084-89.

Nagachinta T, Duerr A, Suriyanon V, et al. (1997). Risk factors for HIV-1 transmission from seropositive male blood donors to their regular female partners in northern Thailand. *AIDS*, 11:1765-72.

Patton DL, Kidder GC, Ganzle GM, Sweeney YC, Clark AM, Hillier SL (1996). Effects of nonoxynol-9 on vaginal microflora and chlamydial infection in a monkey model. *Sex Trans Dis*, 23:461-64.

Patton DL, Sweeney YC, Rabe LK, Hillier SL (1996). The vaginal microflora of pig-tailed macaques and the effects of chlorhexidine and benzalkonium on this ecosystem. *Sex Trans Dis*, 23: 489-93.

Systemic Hormonal Methods for Men

Anawalt BD, Bebb RA, Bremner WJ, Matsumoto AM (1997). Lower dosage levonorgestrel (LNG) and testosterone enanthate (TE): Equally effective spermatogenic suppression and fewer metabolic side effects. 79th Annual Meeting of Endocrine Society, Minneapolis, June 1997, #OR21-6.

Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM (1996). Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: A promising male contraceptive approach. *J Clin Endocrinol Metab*, 81:757-62.

Wang C, Leung A, Superlano L, Steiner B, Swerdloff RS (1997). Oligospermia induced by exogenous testosterone is associated with normal functioning residual spermatozoa. *Fert Steril*, 68:149-53.

Wang C, McDonald V, Leung A, Superlano L, Berman N, Hull L, Swerdloff RS (1997). Effect of increased scrotal temperature on sperm production in normal men. *Fert Steril*, 68, 334-39.

WHO Task Force on Methods for Regulation of Male Fertility

(1996). Contraceptive efficacy of testosterone-induced azoospermia and oligospermia in normal men. *Fert Steril*, 65:821-29.

Wu FCW, Farley TMM, Peregoudov A, Waites GMH, WHO

(1996). Effects of testosterone enanthate in normal men: Experience from a multicenter contraceptive efficacy trial. *Fert Steril*, 65:626-36.

Systemic Nonhormonal Methods for Men (Immunocontraception)

Samaddar M, Catterall JF, Dighe RR

(1997). Expression of biologically active β subunit of bovine follicle-stimulating hormone in the methylotropic yeast *Pichia pastoris*. *Protein Expr Purif*, 10:345-355.

CONRAD-SUPPORTED INVESTIGATORS, 1997

Lois Allen, Ph.D.	Southern Research Institute, <i>Birmingham, AL</i>	Evaluation of the Anti-HIV Activity of Spermicides and Virucides <i>in vitro</i>
Deborah Anderson, Ph.D.	Brigham & Women's Hospital, <i>Boston, MA</i>	Development of Human Cervical and Vaginal Epithelial Cell Culture Systems for Testing Toxicity of Spermicides and Vaginal Microbicides Effects of Contraceptive Methods on Urogenital Tract Inflammation and HIV-1 Titers in Women
Paul Blumenthal, M.D., M.P.H.	Francis Scott Key Medical Center, The Johns Hopkins University, <i>Baltimore, MD</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Lynn Bradley, Lynn, M.S.	Johns Hopkins Medical Service Corporation, <i>Baltimore, MD</i>	A Comparison of the Original FemCap™ and the FemCap™ with Removal Strap: Insertion and Removal
William Bremner, M.D.	Veterans Administration Medical Center, University of Washington, <i>Seattle, WA</i>	Male Contraception: Effects of Desogestrel plus Testosterone Enanthate in Normal Men Effects of Androgens Alone on Maintenance of Severe Oligo/Azoospermia in Men after Induction with a Combination of GnRH Antagonist and Androgen High-Dose GnRH Agonist Effect on Spermatogenesis
George Brode, Ph.D.	Integra LifeSciences Corporation, <i>Plainsboro, NJ</i>	Reproductive Health-STD Control Compositions
Jim Cosentino, Ph.D.	Millersville University, <i>Millersville, PA</i>	Biological Testing of Novel Compounds for Fertility Regulation in the Male
Mitchell Creinin, M.D.	Magee-Women's Hospital, <i>Pittsburgh, PA</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide A Comparison of the Original FemCap™ and the FemCap™ with Removal Strap: Insertion and Removal
Horacio Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva (ICMER), <i>Santiago, Chile</i>	CONRAD Collaborating Center for Clinical Research
Rajan Dighe, Ph.D.	Indian Institute Science, <i>Bangalore, India</i>	Recombinant Expression of Bovine FSH α and FSH β in the Yeast
Bonnie Dunbar, Ph.D.	Baylor College of Medicine, <i>Houston, TX</i>	Immunocontraception Using Zona Pellucida Antigens
Raina Fichorova, M.D., Ph.D.	Brigham and Women's Hospital, <i>Boston, MA</i>	Use of Immortalized Vaginal and Endocervical Epithelial Cells for <i>in vitro</i> Testing of Spermicides and Vaginal Microbicides

Michael Free, Ph.D.	Program for Appropriate Technology in Health (PATH), <i>Seattle, WA</i>	Development of an Easy Fit Silicone Diaphragm
Satish Gupta, Ph.D.	National Institute of Immunology, <i>New Delhi, India</i>	Evaluation of the Efficacy of Recombinant Bonnet Monkey ZP3 and its Corresponding Synthetic Peptides to Regulate Fertility in Primates
S. Marie Harvey, Ph.D.	Pacific Institute of Women's Health, <i>Los Angeles, CA</i>	The Context and Meaning of Reproductive Decision Making Among Inner City Hispanic/Latino Couples
Wayne Heine, M.D.	University of Arizona, <i>Tucson, AZ</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
David Katz, Ph.D.	Duke University, <i>Durham, NC</i>	Targeting Cervical Mucus for Topical Contraceptive and Prophylactic Action Observation and Analysis of Intra vaginal Spreading and Adhesion of Spermicidal/Microbicidal Vehicles
Mark Maltzer, M.D.	Sutter Medical Foundation, <i>Sacramento, CA</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Alvin Matsumoto, M.D.	University of Washington, <i>Seattle, WA</i>	Male Contraception: Progestin-Androgen Combinations Using a Reduced Dose of LNG Plus TE
John Mattox, M.D.	Good Samaritan Medical Center, <i>Phoenix, AZ</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Christopher Miller, D.V.M., Ph.D.	University of California, Davis, <i>Davis, CA</i>	The Heterosexual Transmission of AIDS: A Simian Model
Kenrad Nelson, M.D.	School of Hygiene and Public Health, The Johns Hopkins University, <i>Baltimore, MD</i>	Factors Affecting the Heterosexual Transmission of HIV Infection in Northern Thailand
Alfred Poindexter, M.D.	Baylor College of Medicine, <i>Houston, TX</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Bruce Rosenzweig, M.D.	College of Medicine, University of Illinois, <i>Chicago, IL</i>	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Sungwal Ruggpao, M.D., M.Sc.	Research Institute for Health Sciences, Chiang Mai University, <i>Chiang Mai, Thailand</i>	DMPA Use and HIV Transmission in Northern Thailand
Anthony Sacco, Ph.D.	Wayne State University, <i>Detroit, MI</i>	Evaluation of the Efficacy of Synthetic Peptides Corresponding to Primate/Human ZP3 to Regulate Fertility in Primates
Haleh Sangi-Haghpeykar, Ph.D.	Baylor College of Medicine, <i>Houston, TX</i>	Psychosocial Predictors of Condom Use among Injectable Contraceptive Users

Alfred Shihata, M.D.	FemCap™, Inc., Del Mar, CA	Clinical Evaluation of Femcap®
Ronald Swerdloff, M.D.	Harbor-UCLA Medical Center, Torrance, CA	Effects of Androgens Alone on Maintenance of Severe Oligo/Azoospermia in Men after Induction with a Combination of GnRH Antagonist and Androgen High-Dose GnRH Agonist Effect on Spermatogenesis
Derek VanAmerongen, M.D.	Johns Hopkins Medical Service Corporation at Wyman Park, Baltimore, MD	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Anne Viselli, M.D.	Vermont Women's Health Center, Burlington, VT	Safety and Efficacy of FemCap™ Used with Spermicide and the Ortho All-Flex® Diaphragm Used with Spermicide
Christina Wang, M.D.	Harbor-UCLA Medical Center, Torrance, CA	Comparison of the Efficacy of a Progestagen Implant (Norplant® II) in Combination with Transdermal Androgen versus Transdermal Androgen Alone in Suppression of Spermatogenesis in Normal Men
Fred C.W. Wu, M.D., F.R.C.P.	University of Manchester, Manchester, England	Effects of Oral Desogestrel Intramuscular Testosterone in Normal Men: A Pharmacokinetic and Pharmacodynamic Study
Lourens Zaneveld, Ph.D.	Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL	Identification, Evaluation and Development of Contraceptive Antimicrobials

CICCR-SUPPORTED INVESTIGATORS, 1997

David Abbott, Ph.D.	Wisconsin Regional Primate Research Center, Madison, WI	Effect of Pure Extract of Triptolide from <i>Tripterygium wilfordii</i> on the Fertility of Male Marmoset Monkeys, <i>Callithrix jacchus</i>
Vichai Reutrakul, Ph.D.	Mahidol University, Bangkok, Thailand	
Mitchell Avery, Ph.D.	University of Mississippi, University, MS	Inhibition of Lactate Dehydrogenase-C ₄ as a New Approach to Male Contraception
Ben Kunlong, Ph.D.	Chinese Academy of Science, Kunming, Yunnan, P.R. China	Effects of IgA Antibody Against Sperm-Specific Antigen LDH-C ₄ on <i>in vivo</i> Fertilization of Balb/c Mice
William J. Bremner, M.D., Ph.D.	VA Puget Sound Health Care System, Seattle, WA	Levonorgestrel Implant Study in Shanghai
James F. Catterall, Ph.D.	The Population Council, New York, NY	Structure and Function of the FSH Receptor: A Potential Immunocontraceptive
C. Yan Cheng, Ph.D.	The Population Council, New York, NY	Induction of Release of Premature Germ Cells from Seminiferous Epithelium by Analogs of Lonidamine

James Cosentino, Ph.D.	Millersville University, <i>Millersville, PA</i>	Testicular Dihydrofolate Reductase: Preliminary Isolation, Characterization and DNA Expression
Patricia Cuasnicu, Ph.D.	Instituto de Biología y Medicina Experimental, <i>Buenos Aires, Argentina</i>	Potential Contraceptive Use of an Epididymal Protein
Jacqueline E. Darroch, Ph.D.	The Alan Guttmacher Institute, <i>New York, NY</i>	Balancing Concerns: Assessing Women's Potential Interest in Using Microbicides
Bonnie S. Dunbar, Ph.D.	Baylor College of Medicine, <i>Houston, TX</i>	Identification of Human ZP Peptides Involved in Sperm-Zona Interaction
Fernando Larrea, M.D.	Instituto Nacional de al Nutricion Salvador Zubiran (INNSZ) <i>Mexico D.F., Mexico</i>	
Anibal Faundes, M.D.	Centro de Pesquisas das DoenÇas, Materno-Infantis de Campinas (CEMICAMP), <i>São Paulo, Brazil</i>	Studies of Tolerance and Spermicidal Effect of a Bio-adhesive Acidform Gel Containing Nonoxynol-9
Frank French, M.D.	University of North Carolina at Chapel Hill, <i>Chapel Hill, NC</i>	Human Epididymal Protein Targets for Male Contraception
Geula Gibori, Ph.D.	University of Illinois at Chicago, <i>Chicago, IL</i>	Use of a 20-hydroxysteroid Dehydrogenase as a Potent Contraceptive
David Handelsman, Ph.D.	Royal Prince Alfred Hospital Medical Centre, University of Sydney, <i>Sydney, Australia</i>	The Efficacy and Safety of Low Dose Estradiol Supplementation for Depot Testosterone-Induced Suppression of Human Spermatogenesis
Matthew Hardy, Ph.D.	The Population Council, <i>New York, NY</i>	Suppression of the Pituitary-Gonadal Axis in the Primate by a Synthetic Androgen, 7 α - methyl-19-nortestosterone, Alone and in Combination with Estradiol
A. Jagannadha Rao, Ph.D.	Indian Institute of Science, <i>Bangalore, India</i>	
John C. Herr, Ph.D.	University of Virginia, <i>Charlottesville, VA</i>	Formulation and Testing of a Native S19 Monoclonal Antibody/Novasome [®] Spermicidal Cream
Anil Suri, Ph.D.	National Institute of Immunology, <i>New Delhi, India</i>	Isolation and Characterization of Genes Encoding Sperm Surface Antigens by Screening Human Testis Expression cDNA Library: Identification of a Candidate Molecule(s) for Development of Contraceptive Vaccine
Gabor Huszar, M.D.	Yale University, <i>New Haven, CT</i>	Sperm Creatine Kinase M-Isoform Assay for the Assessment of Residual Fertility in Men Treated with Suppressants of Spermatogenesis
Stephen Killick, M.D., F.R.C.O.G.	Princess Royal Hospital, <i>Hull, England</i>	The Contraceptive Action of Progestogens on Cervical Mucus

Cristina Meriggiola, M.D.	S. Orsola Hospital, University of Bologna, <i>Bologna, Italy</i>	Effects of a Sequential Regimen of Cyproterone Acetate and Testosterone Undecanoate Followed by Lower Dose Cyproterone Acetate and Testosterone Undecanoate in Normal Men
Michelle M. McKenna	FEI Technologies, Inc., <i>Plainsboro, NJ</i>	Contraceptive Drug Delivery Systems Development
William Miller, Ph.D.	North Carolina State University, <i>Raleigh, NC</i>	Effects of GnRH Antagonists upon Human Sperm-Zona Pellucida Binding
Patricio Morales, Ph.D.	Instituto de Medicina Reproductiva (ICMER), <i>Santiago, Chile</i>	
Lois Salamonsen, Ph.D.	Prince Henry's Institute of Medical Research, Clayton, <i>Victoria, Australia</i>	Inhibition of Embryo Implantation by Inhibitors of Matrix Metalloproteinases
Gerald Schatten, Ph.D.	Oregon Regional Primate Research Center, <i>Beaverton, OR</i>	Disintegrin-Integrin Involvements during Sperm-Oocyte Binding in Primates: A Novel Binding Site for Designing Contraceptive Strategies
Horacio Croxatto, M.D.	Instituto Chileno de Medicina Reproductiva (ICMER), <i>Santiago, Chile</i>	
Gao Er-Sheng, M.D., M.P.H.	Shanghai Institute for Planned Parenthood Research <i>Shanghai, P.R. China</i>	
Robert Sullivan, Ph.D.	Laval University, Ste-Foy, <i>Quebec, Canada</i>	P34H: A Human Sperm Immunogen with an Immunocontraceptive Potential
Pedro Verdugo, Ph.D.	University of Washington, <i>Seattle, WA</i>	Control Mechanisms of Swelling and Rheology of Human Mucus
Manuel Villalon, Ph.D.	Instituto Chileno de Medicina Reproductiva (ICMER), <i>Santiago, Chile</i>	
Harold Verhage, Ph.D.	University of Illinois at Chicago, <i>Chicago, IL</i>	Immunocontraceptive Potential of an Oviduct-Specific Glycoprotein
Yang Zengming, Ph.D.	Northeast Agricultural University, <i>Harbin, P.R. China</i>	Leukemia Inhibitory Factor and Implantation in Monkeys
Lourens Zaneveld, Ph.D.	Rush-Presbyterian-St. Luke's Medical Center, <i>Chicago, IL</i>	Polystyrene Sulfonate for Vaginal Contraception and Disease Prevention Pre-IND Development of Cellulose Sulfate as a Contraceptive Antimicrobial