

Methods for Men

An ideal male contraceptive must not only be highly effective, but must also produce minimal adverse effects and be acceptable, suitable, and affordable to men in both developed and developing countries. The most advanced studies involve regimens of androgen/progestin combinations that suppress gonadotropins, thereby blocking sperm production, but other approaches continue to be investigated. Male methods remain one of CICCRR's research priority areas.

SYSTEMIC HORMONAL METHODS

The first male method to reach fruition will likely involve a hormonal combination, given that such combinations have already reached the stage of clinical testing. Androgen/progestin combinations have been shown to induce azoospermia and significant oligospermia more quickly than androgens alone. Progestins may also permit the use of less testosterone in the regimen, thereby reducing side effects such as acne, weight gain, or adverse effects on serum lipids, especially high density lipoprotein (HDL) cholesterol. None of the regimens currently under investigation appears to be ideal, but ongoing studies will direct research toward new and better methods.

One of the most promising studies involved a combination of the antiandrogenic progestin, cyproterone acetate (CPA), and testosterone undecanoate (TU). In this study, a high percentage of men reached azoospermia after approximately 10 weeks of treatment, and the remainder were severely oligospermic and would likely have been infertile. However, the producer of these agents expressed reservations about further development of this combination because of concern that long-term exposure to CPA might cause unanticipated side effects for men.

Norethisterone (NET) enanthate plus TU is being considered as an alternative regimen and has the advantage of requiring only one injection every 8 weeks. CONRAD plans to collaborate with WHO on a large-scale multicenter efficacy study, but indemnification, liability, and patent issues must first be resolved.

Final data analyses are almost complete for a study to determine the lowest oral dose of the synthetic progestin levonorgestrel (LNG) used in combination with testosterone enanthate (TE) that will suppress spermatogenesis. During earlier phases of the study, doses of 125-500 µg LNG/day induced significant inhibition of spermatogenesis, but also modest reduction of HDL cholesterol. So far, analyses show that doses of 63 and 32 µg LNG/day, in combination with TE, yield similar results, with severe oligospermia in essentially the same percentage of men and in the same time frame. Rates of azoospermia and decreases in HDL levels were equivalent to those in men who received 125 µg LNG/day.

A previous study evaluating a combination of two Norplant®-II systems (4 rods) containing LNG and two testosterone (T) patches resulted in inadequate sperm suppression. Thus, the study was expanded to include two additional groups of men: one group receiving four Norplant-II rods plus weekly TE and another group receiving daily T patches plus oral LNG daily. Preliminary results indicate that Norplant-II rods plus weekly TE suppresses sperm production significantly more than T patches plus oral LNG. However, HDL-cholesterol levels were decreased more in the Norplant-II plus TE group.

A two-center study to test the combination of depot testosterone plus depot medroxyprogesterone acetate (DMPA) is nearing completion in Australia. Symptomatic androgen deficiency noted

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in a few men early in the study required increasing the frequency of T pellet replacement. In addition, DMPA injections were eliminated from the second half of the treatment phase because circulating MPA levels remained longer than expected. Of the 56 men enrolled, 27 discontinued—15 for treatment-related reasons—suggesting that acceptability concerns may prevent widespread use of this combination. Many of the discontinuations were due to extrusion of the T pellets, consistent with previous rates. However, the regimen successfully suppressed sperm production and there were no pregnancies, confirming previous preliminary studies of this combination.

A study examining the combination of DMPA plus TU in tea seed oil showed that TU alone does not reliably induce azoospermia in all subjects. All men receiving combinations of TU and DMPA reached azoospermia and maintained full spermatogenic suppression until the end of the treatment period. However, spermatogenic rebound occurred slowly. Given these results and those from the study in Australia, a change in the injection interval or dose of DMPA may be warranted, as the recovery of spermatogenesis seems unduly delayed with the present DMPA-containing regimens.

Another study of T pellets with or without four rods of Norplant-II is being conducted in Chinese versus non-Asian men. The Chinese component is being carried out in Nanjing using Twinning funds. All investigators received training in T pellet insertion.

A study to evaluate a combination of two products made in China, TU in tea seed oil with and without Sinoplant, an equivalent of Norplant-II, is underway. Most men receiving both hormones became azoospermic or experienced sperm suppression of less than 3 million/ml. No

instances of altered libido or sexual function have been noted.

The Chinese TU formulation is only half the concentration of that produced by its European manufacturer and, consequently, large volumes (8 ml/injection) are needed, which would likely adversely influence acceptability. Thus, the manufacturers are reformulating TU in soybean oil to achieve the higher concentration. Although the factory appears to meet Good Manufacturing Practice standards, obtaining certification from the FDA would require additional resources.

Given the need for improved androgen preparations, a Phase I safety study is evaluating a new T microsphere formulation in normal men. If the study is successful and the formulation acceptable, a follow-up study will compare T microspheres alone versus T microspheres plus either two systems of Norplant-II or intramuscular injections of DMPA, and should provide enough information to support proceeding to a clinical efficacy study in the United States.

Additional studies have been conducted in primates to understand the intratesticular mechanisms underlying hormonal control of spermatogenesis. Such studies may help determine what hormonally based contraceptive regimens will lead to azoospermia in the highest percentage of men. Monkeys were administered testosterone alone or testosterone plus either LNG or CPA. Gonadotropin suppression, particularly follicle stimulating hormone (FSH), more closely correlated with ultimate suppression of sperm production in the monkeys than in men, though variable degrees of spermatogenesis suppression occurred in each of the three groups.

SYSTEMIC NONHORMONAL METHODS

Antitesticular Agents

Lonidamine is an anticancer drug developed in the 1970s with demonstrated antispermatogenic activity. However, development as an antispermatogenic agent was abandoned in the 1980s due to kidney damage caused by high doses. Analogs are currently being screened to identify those equally effective but non-toxic. Two compounds, designated AF-2364 and AF-2785, show specific effects in depleting immature germ cells from the seminiferous epithelium. Preliminary immunohistochemistry and animal studies revealed that these compounds appear to exert their effects only within the testis, without affecting other reproductive organs, and have no apparent effects on mature sperm already in the epididymis. Weekly dosing of AF-2364 in rats resulted in reversible infertility. Genotoxicity studies, including mutagenicity, were negative, as were acute toxicology studies.

Two-week rat and mouse toxicity studies, not funded by CONRAD, will begin in 2002. In the meantime, reversibility studies and synthesis of new analogs will continue, and an efficacy study in male marmosets has been funded by CICC to provide proof of concept in a primate. An Italian biotechnology company is serving as the industrial partner in this project.

Epididymal Proteins

Sperm passing through the epididymis mature and acquire fertilizing capacity, suggesting post-testicular modification of the sperm by epididymal proteins and/or luminal fluid. A major challenge is to identify proteins expressed only in the epididymis also present on sperm. Human Genome Sciences (HGS) has prepared human epididymal gene libraries for automated sequencing, and several clones are currently under investigation for androgen

regulation, epididymal specificity, sperm binding, and functional relevance.

Preliminary results suggest that there are at least two or three potential targets that should be vigorously pursued. Future work will involve developing peptides or other agents that can inhibit these proteins. Monkey contraceptive studies for the already identified target antigens and characterization of newly received clones will continue.

Male monkeys were immunized in Bangalore, India, using several of the HGS-identified expressed proteins as well as the human homolog of acidic epididymal glycoprotein (AEG), and the effects on androgen levels and sperm quality and production are being monitored. Previous studies in rats indicated that AEG is involved in fertilization and represents a potential immunocontraceptive target for men and women. In the monkeys immunized, serum testosterone levels were not affected. Monkeys immunized with Clone 23 (Eppin, an epididymal protease inhibitor) generated good antibody titers and were mated. Baboons also have been immunized with recombinant Eppin in Kenya and monitored for effects on sperm counts, testosterone levels, sperm motility, and fertility. Experiments to test the effects of Eppin on sperm-egg interaction are planned. Additional proteins under investigation in Bangalore include LCN6 (a lipocalin), HE2a, HE2b, and ESC42. Substantial antibody titers have been obtained with each of these proteins, and fertility testing is underway.

Testis- and Sperm-Specific Targets

A potent antiestrogen has been shown to cause infertility in male rats. Dose-response and reversibility studies with this compound are being planned in dogs. If these are successful, contraceptive trials in a nonhuman primate will be required.

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Other potential targets are testis-specific enzymes. Isoforms of the detoxification enzyme glutathione S-transferase (GST) are present in the seminiferous tubule fluid and on the sperm surface. Inhibition of GST using glutathione analogs leads to interference of sperm function, such as motility, acrosome reaction, and fertilizing ability. Human soluble adenylyl cyclase appears to be involved with the signal transduction events in the sperm during capacitation, hyperactivation, and the acrosome reaction. Testis-specific expression of the novel enzyme would open up opportunities to develop sperm-specific antagonists of its activity.

HIV/STI Prevention and Epidemiology Studies

In 2001, 40 million people were living with HIV/AIDS and 5 million were newly infected. Preventing the spread of this epidemic is a top research priority for CONRAD. In addition to support for research and development of microbicides and barrier methods, CONRAD, with funds from the CDC, is also supporting epidemiology and prevention studies.

Highly active antiretroviral therapy (HAART) is a form of AIDS treatment that improves immunological parameters in the peripheral blood. Significant differences in T cell numbers and receptor repertoires have been found between cervical and peripheral sites at different disease stages and between cervical and other mucosal sites. These differences may reflect both normal phenotypic differences as well as differences in pathogenic effects of HIV infection. Statistical approaches to the question of whether HAART reconstitutes the cervical T cell and antibody repertoire (and, if so, whether to the same

extent as in peripheral blood) are being devised in collaboration with the CDC. Consistent use of condoms with DMPA is particularly important due to the sharp rise in the incidence of HIV infection and other STIs among reproductive age women. An ongoing study seeks to identify psychosocial factors that best predict use of condoms among injectable contraceptive users.

Studies examining the acceptability and feasibility of using and advocating the use of diaphragms for prevention of STIs are also underway. The objectives are to determine whether women in Zimbabwe who are unsuccessful male or female condom users are willing to use a diaphragm with lubricant, to examine compliance with and acceptability of diaphragms among women, and to determine whether a large intervention trial, examining the effectiveness of diaphragms with a chemical barrier in preventing HIV and other STIs, is a feasible and desirable next step in this study population. Also, a randomized clinical trial in Kenya is trying to determine whether the diaphragm used with K-Y[®] Jelly is effective in preventing *Neisseria gonorrhoeae* and *Chlamydia trachomatis* reinfection in women.

There is little published data on the rate of HIV progression among individuals infected with HIV-1 subtype E, which is common in Southeast Asia. In Thailand, factors associated with HIV progression are under investigation. Morbidity, changes in CD4+ lymphocyte counts and HIV viral load, and the incidence of HIV infection among initially HIV-negative women are being assessed.

The prevalence of bacterial vaginosis (BV) and other conditions affecting women's reproductive health is poorly documented in Azerbaijan. A prior CDC study provided estimates of the prevalence of BV in

rural Azerbaijan. A new study has been initiated to identify sociobehavioral and demographic risk factors associated with BV. Previously used diagnostic tests will be compared with Gram stain analysis to provide additional important information on the sensitivity and specificity of these tests, verify the prevalence of BV in this community, and assess the accuracy of syndromic diagnosis and rapid tests not requiring microscopy.

A pending study will analyze and document the risk of HIV infection in Italian women who have been previously inseminated after their HIV-positive partner's semen has been processed by a specific sperm-washing method. Absence of infection in children conceived by this method will be documented as well. A questionnaire will be used to evaluate how the availability of this procedure has altered the sexual behavior of the couple, especially with regard to practices with a high risk of HIV transmission. Several different sperm-washing techniques will also be evaluated in an accompanying lab study.

Methods for Women

This area of research aims to expand technologies available to women that would protect them from pregnancy and STIs. This is a key objective, not only for CONRAD, but also for USAID, CONRAD's primary funding agency and closest collaborator. In addition, Family Health International (FHI) provides essential services in the areas of data management and biostatistics to help expedite the development of new methods.

CHEMICAL BARRIERS

Vaginal spermicide/microbicide formulations are a promising approach to preventing both unwanted pregnancies and STIs. The Joint United Nations Programme on

HIV/AIDS (UNAIDS) COL-1492 study demonstrated that use of a nonoxynol-9 (N-9) preparation, which was expected to prevent HIV infection, might actually have increased its incidence in a high-risk population. This could have been due to the vaginal irritation associated with N-9. Thus, the need to find a nonirritating agent is greater than ever.

Such a method also should be woman-controlled; provide effective levels of drug at the target site; minimize systemic exposure to the active ingredients, thereby minimizing adverse drug effects; and promote drug distribution and retention in the vaginal vault and over the cervix. The ideal product is one that is not messy, does not leak from the vagina, coats the whole vaginal surface rapidly, and has a prolonged action of at least 12 hours. The prolonged action would ensure that it could be used privately by the woman well ahead of the time when it is needed.

Preclinical Testing

CONRAD's intramural preclinical program evaluates the antifertility and antimicrobial activity of new compounds and formulations and assists in development of selected candidates for microbicides and contraceptives. Characterization of contraceptive activity is performed through multiple tests ranging from *in vitro* spermicidal assays to rabbit fertility trials, typically following an established algorithm. Activity against HIV and other sexually transmitted pathogens is assessed through this program as well as through collaborating laboratories. To further expedite this process, CONRAD is establishing a network of investigators to evaluate agents shown to have good anti-HIV activity *in vitro* for their activity against other sexually transmitted pathogens using *in vitro* methods and animal models. Support will also be provided to help improve or expand testing technology.

Vaginal formulations are a promising approach to preventing unwanted pregnancies and STIs. Several compounds now show promise as potential microbicides and/or contraceptives.

Of the microbicide leads supported by CONRAD, the one furthest along in development is cellulose sulfate. GMP is working with outside contractors for large-scale drug synthesis and clinical supply production for future studies.

Several compounds identified through pre-clinical screening now show promise as potential microbicides and/or contraceptives, including a naphthyl urea derivative and two acylcarnitine analogs (Z-14 and Z-15). Further characterization of additional CONRAD leads is also continuing.

The first phases of a project to genetically modify selected strains of lactobacilli to secrete human CD4 or express it on the cell surface have been completed. The presumption is that the modified lactobacilli would efficiently colonize the reproductive tract, and the enhanced levels of CD4 at the mucosal surface would trap HIV, thereby reducing infection. The investigators have succeeded in producing lactobacilli that secrete CD4 that can reduce HIV infection in cell culture systems in a dose-dependent manner.

CONRAD is also contributing to further development of UC-781, a novel non-nucleoside reverse transcriptase inhibitor. Initial phases include completing the development of a stable bioactive formulation and conducting additional toxicology studies.

Clinical Assessment of Lead Products

Of the microbicide leads supported by CONRAD, the one furthest along in development is cellulose sulfate (CS). Two clinical safety studies are complete, and GMP is working with outside contractors for large-scale drug synthesis and clinical supply production for future studies. The clinical development plan constructed for CS is based on guidelines established by the International Working Group on Microbicides (IWGM).

- Two safety studies have been completed: a 6-day Phase I safety study of CS gel compared to Conceptrol® and K-Y Jelly in healthy women, and a 7-day male tolerance study of CS gel compared to Conceptrol in both circum-

cised and uncircumcised men. Both studies showed that CS was safe and as acceptable as the marketed products.

- In collaboration with the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, and WHO, an expanded safety study of CS in Uganda, Nigeria, and India has begun enrollment and is expected to be completed late in 2002.
- The following safety studies are also being planned: in collaboration with the HIV Prevention Trials Network, a safety study expected to start in spring 2002 of HIV-infected women; a 14-day expanded safety study of women in the United States; a safety study of rectal use in men and women; and a safety and acceptability study of four applications per day in sexually active women in Cameroon.
- Preparation for Phase II/III prevention studies for HIV and other STIs and contraceptive efficacy studies was initiated in 2001, including discussions with the FDA and other collaborating agencies, such as FHI, WHO, and ITM. The current plan is to begin these studies by the end of 2002, but this depends on the satisfactory completion of the ongoing toxicology studies, preparation of ample quantities of clinical supplies, recruitment of the required clinical sites, and receipt of adequate funding.

A Phase I safety study of polystyrene sulfonate (PSS) is complete, the results of which suggest that PSS is associated with less genital irritation than the marketed N-9 product, Conceptrol. Contractors meeting Good Manufacturing Practice guidelines have been selected to produce drug substance and clinical supplies for future studies. It is anticipated that by the fall of 2002, adequate material meeting these guidelines will be available to start

an expanded Phase I safety study in women and a male tolerance study, as well as to initiate long-term toxicology studies.

ACIDFORM is a vaginal gel with high buffering capacity that maintains vaginal pH below 4.5 even after seminal fluid deposition. A safety study in Brazil found an ACIDFORM formulation with N-9 highly irritating to the vagina, whereas ACIDFORM without N-9 showed no evidence of irritation. Since ACIDFORM is thought to have better buffering capability than BufferGel (a similar product moving into Phase III testing) and appears to be bioadhesive, further research will explore its contraceptive and antimicrobial properties.

- A postcoital study is nearly complete. Women received three cycles of treatment: ACIDFORM inserted 0-2 hours before intercourse, ACIDFORM inserted 8-10 hours before intercourse, and N-9 inserted 0-2 hours before intercourse. Data analysis is ongoing, but preliminary results suggest that ACIDFORM could provide effective contraceptive activity for at least 10 hours.
- Because the original safety study in Brazil only involved a small number of women, a 14-day expanded Phase I safety study in the United States is being planned in which women would use the product twice a day with and without intercourse. Both the expanded safety study and a male tolerance study are expected to start in the summer of 2002 following submission of an investigational new drug application.
- Previous studies in Brazil used batches of gel prepared in Brazil by the product's developer according to FDA Good Manufacturing Practice guidelines. In preparation for U.S. clinical studies, a U.S. contractor meeting these guide-

lines has been selected to produce future batches.

A GMP-funded male tolerance study of BufferGel versus K-Y Jelly is now complete. BufferGel was found to be safe, though one in eight users experienced genital symptoms, such as itching and burning, for a brief time after penile application.

Clinical development of PRO 2000 gel has been underway since 1996 and has generally shown to be safe and well tolerated in women. Results from a Phase I study completed by CONRAD in 2001 also suggest that PRO 2000 is safe in men. One in six users of both the active and placebo gel reported mild, transient genital symptoms such as itching and tingling, and PRO 2000 was undetectable in plasma samples taken after seven consecutive days of exposure. Additional pre-clinical toxicology testing is underway with CONRAD/ GMP funding.

CONRAD is also supporting development of C31G, a mixture of two amphoteric surface-active compounds with broad-spectrum activity. Dose escalation and postcoital testing studies of three concentrations, 0.5%, 1.0%, and 1.7%, were recently completed. Based on the results of these studies, a male tolerance study using 1.0% C31G is planned for the spring of 2002.

Additional Contributions to the Field
In addition to drug research and development, CONRAD is supporting other efforts to move the field of chemical barriers research forward. A study is underway to compare three techniques used to evaluate product spreading in the vagina: a fiber optic probe to image fluorescein-labeled products; gamma-scintigraphy, which uses radio-labeled material; and magnetic resonance imaging (MRI), which detects gadolinium-labeled material.

Colposcopy has been used increasingly in the development of vaginal products to detect epithelial changes invisible to the naked eye that may increase the likelihood of HIV infection or other STIs.

Another study to evaluate CS using only MRI is also underway.

Currently, there are a limited number of suitable sites with trained personnel in high HIV-incidence areas to undertake proposed HIV-prevention studies in a timely manner. In collaboration with ITM and WHO, CONRAD is working to identify and develop clinical sites for these studies and to ensure adequate training of investigators, clinical monitors, and laboratory staff.

Colposcopy has been used increasingly in the development of vaginal products to detect epithelial changes that are not visible to the naked eye and that may increase the likelihood of HIV infection or other STIs. In collaboration with IWGM and in association with UNAIDS, CONRAD convened a meeting that resulted in a revision of WHO's "Manual for the Standardization of Colposcopy for the Evaluation of Vaginally Administered Products." CONRAD's clinical site in Norfolk, VA, has trained a number of investigators from other sites to carry out colposcopy according to the revised manual.

MECHANICAL BARRIERS

Lea's Shield

Lea's Shield is a cup-shaped barrier device made of silicon rubber with a valve that allows venting of air trapped between the cervix and the device and a loop that aids in insertion and removal. Two clinical studies have been completed, both of which were requested by the FDA to support a PMA submission. One found modest colposcopic and microbiological changes in the vagina and cervix after 8 weeks of use. No clinical infections were diagnosed during the study, and some partner discomfort was noted. An MRI study in two women confirmed that Lea's Shield completely covers the cervix as

described by the developer. A PMA was submitted in the fall of 2001.

FemCap

FemCap is a silicone rubber cervical cap that comes in three sizes. A Phase II/III contraceptive efficacy trial of this device compared with the Ortho All-Flex™ diaphragm showed that, while the two devices were not equivalent in contraceptive efficacy as defined by the study's hypothesis, both had associated pregnancy rates within the expected range for barrier methods. However, significantly more FemCap users reported problems with removal and dislodgment.

To address these problems, the developer modified the device by adding a removal strap and increasing the height of the brim in the medium and large sizes. An ad hoc study of some of the women who had reported trouble with device removal in the Phase II/III trial found that significantly fewer women had trouble removing the strapped device.

However, a longer safety and acceptability study showed that the modifications did not significantly improve ease of device removal. In addition, there were significantly more reports of participant and partner pain/discomfort and decreased acceptability in the group using the strapped device. In the developer's opinion, couples experiencing problems associated with removal, pain, and discomfort will likely not continue its use, and while not acceptable for all users, FemCap would expand the contraceptive choices for those who choose to use it. The developer has decided to submit a PMA for the strapped device, and CONRAD will assist by writing the clinical safety and efficacy sections.

Female Condoms

CONRAD is also supporting efforts to develop an improved female condom. The Program for Appropriate Technology in

Health (PATH) is developing a device based on user feedback and an iterative design process. An acceptability study of the first prototype is expected to start in early 2002.

Three acceptability and functional performance studies for the natural latex Reddy female condom have been completed. The design continues to be modified to address slippage issues. New devices will undergo a quality analysis and, if they prove to be durable, CONRAD will support a comparative acceptability and functional performance evaluation in the United States and a larger trial at two centers in India.

SILCS Intravaginal Barrier Device
PATH is also developing a reusable, one-size device in conjunction with SILCS, Inc. A Phase I postcoital testing and safety study comparing the device with the Ortho All-Flex diaphragm showed that, although motile sperm was found in only one postcoital testing cycle, a few women experienced some difficulties handling and using the PATH/SILCS device. Investigators from PATH concluded that the design should be modified to accommodate a better fit in a broader range of women. New prototypes are being produced, and CONRAD will collaborate with PATH to conduct a Phase I couple study of the modified device when it becomes available.

BufferGel Cup
This device is designed to efficiently deliver microbicide/spermicide on both the device's cervical and vaginal side. Design variations of the prototype are being explored as are improved fabrication methods. Manufacturing capabilities were established and pilot injection molded devices are being produced.

SYSTEMIC HORMONAL METHODS

Progestin Delivery Systems

An LNG-releasing, single-rod implant was compared to the marketed Norplant system in a Phase I study. The single rod produced lower serum levels of LNG and less favorable bleeding patterns than the Norplant system. However, ovulation patterns were not different between the two groups and the risk of pregnancy would be expected to be about the same. A single-rod implant should enable easier insertion and removal, thereby making it more affordable. However, the duration of effect would be shorter (e.g., 1 year) and may impact the acceptability of the product. An industrial partner would be needed for this method, as the current manufacturer has decided not to pursue this approach.

Emergency Contraceptives

There are two widely available methods of emergency contraception, LNG alone (0.75 mg orally, 12 hours apart) or combined with ethinyl estradiol (0.5 mg LNG plus 0.1 mg ethinyl estradiol orally, 12 hours apart: the Yuzpe method), both of which are relatively effective. CICCR is supporting efforts to understand the mechanisms of action involved in these methods in order to decrease the failure rate or expand the window of efficacy.

A two-center study in Chile and the Dominican Republic suggests that when the size of the follicle is less than 18 mm at the time of treatment, the efficacy of the Yuzpe method is due to inhibition of follicular rupture. When the follicle size is greater than 18 mm, ovulation is not inhibited, and so other mechanisms of action might come into play. This failure to inhibit ovulation could provide an explanation for the overall 25% failure rate of this method.

Because the LNG alone treatment is more effective than the Yuzpe method, it is

Three acceptability and functional performance studies for the Reddy female condom have been completed. The design continues to be modified to address slippage issues.

A Phase II trial of an emergency contraceptive regimen was designed to increase the window of efficacy. Women received either mifepristone, an antiprogestin, plus tamoxifen, an antiestrogen, or mifepristone plus a placebo.

important to determine whether the same phenomenon of inhibition of follicular rupture is the major explanation for its efficacy. Such a study has been completed, and results indicate that, as with the Yuzpe regimen, a considerable part of the contraceptive effect is due to inhibition of follicular rupture.

A Phase II trial of an emergency contraceptive regimen completed in China was designed to increase the window of efficacy. The hypothesis was that an antiprogestin/antiestrogen combination would delay endometrial development and could thus prevent implantation if treatment occurred too late to inhibit ovulation. Women received either mifepristone, an antiprogestin, plus tamoxifen, an antiestrogen, or mifepristone plus a placebo. Results indicate no significant increase in pregnancies when treatment was delayed for up to 5 days after unprotected intercourse with either regimen. The addition of tamoxifen seemed to improve efficacy, but the difference between regimens was not significant. A large-scale study will be necessary to assess whether this trend is indeed significant.

In 2001, the CICCRR Strategic Advisory Board recommended that CICCRR not support such a study unless significant support from pharmaceutical companies was forthcoming. The advantage of mifepristone alone for emergency contraception is that only one pill is needed. The addition of tamoxifen would necessitate taking two pills, but still only at one time. Now, the most effective approved treatment is with LNG, but this regimen requires taking two pills 12 hours apart. An ongoing pharmacokinetic and an emergency contraceptive study suggest that, in fact, the total LNG dose can be taken immediately with no loss of efficacy. CICCRR will await the outcome of these current studies before deciding to initiate any other studies. A multicenter study of mifepristone plus

tamoxifen is likely to be expensive and not possible with existing CICCRR funding.

Sequential Antiprogestin/Progestin Regimen

Investigators in Chile have previously studied a sequential regimen of an antiprogestin, mifepristone, on days 1-15 of the cycle followed by a progestin, medroxyprogesterone acetate, on days 16-28 as an improved method for inhibiting ovulation. Results from this study show that ovulation still occurred in many of the cycles, but that the rise in plasma luteinizing hormone and follicular rupture took place during the period of treatment with the progestin. To improve efficacy of this method for inhibiting ovulation, the mifepristone dose was increased, and the progestin used was changed to norgestrel acetate, which has potent antigonadotropic activity. With this regimen given over three cycles, ovulation only occurred in a few cycles, and in those ovulatory cycles the luteal phase was defective, which would reduce the chance of pregnancy. Data from a Phase II contraceptive efficacy trial is under analysis.

SYSTEMIC NONHORMONAL METHODS

Development of systemic nonhormonal methods for women remains a difficult area of research. Thus, projects in this area are at early feasibility stages. In addition, the hopes for successful development of an immunocontraceptive for women have diminished recently as results from primate trials by several investigators using a variety of immunogens have been less than encouraging.

For example, several investigators targeted antigens of the zona pellucida (ZP), the extracellular envelope surrounding the egg. ZP proteins mediate the initial binding of sperm and subsequent activation

events during fertilization. Immunization of various female animals with ZP glycoproteins lead not only to blocks in fertility, but often to disturbances in cyclicity, hormonal profiles, and folliculogenesis. However, investigators in India conducted immunogenicity studies in a bonnet monkey model using ZP1 epitopes, which did not induce ovarian disruption but did significantly reduce fertility in a small group of monkeys. Other investigators focused on *in vitro* methods to predict *in vivo* immunogenicity in primates and the use of oligonucleotide probes directed against sperm antigens to disrupt sperm-egg interactions.

Other antifertilization approaches included the characterization of an FSH-binding inhibitor (FSHBI) purified from ovarian follicular fluid that acts by inhibiting progesterone levels. About half of the marmosets treated with an FSHBI peptide to assess inhibition of folliculogenesis and luteinization experienced impaired fertility.

In addition to cosponsoring some of the projects mentioned above, CICCRR has also supported monthly methods research targeting gonadotropin-releasing hormone action, signaling pathways such as angiogenesis, and other cytokines/growth factors that appear essential for implantation, such as interleukin-11 and leukemia inhibitory factor. Because the biological processes involved in these signaling pathways are not well understood, it remains a difficult area of study. It is hoped that once an approach has proven successful, there will be more collaborative interest from the for-profit sector. Antifertility studies in monkeys have been funded for many of the leads, the results of which will be the basis for future project decisions.

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Systemic Hormonal Methods for Men

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