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WHO Special Programme of Research, Development and Research Training in Human Reproduction

Launched by the World Health Organization in 1972, the Special Programme of Research, Development and Research Training in Human Reproduction is a global programme of technical cooperation. It promotes, coordinates, supports, conducts, and evaluates research on human reproduction, with particular reference to the needs of developing countries. In May 1988 UNDP, UNFPA, the World Bank, and WHO became joint co-sponsors of the Programme.

1992
20th Anniversary of the Programme

INSIDE

Programme's contraceptive research and development: highlights of 1991

New injectable contraceptives poised for a bigger role

Injectable contraceptives are very effective, and they need to be administered only once every 4–12 weeks. The most widely used preparations are depot-medroxyprogesterone acetate (DMPA) and norethisterone enantate (NET-EN), which are administered every three and two months, respectively. Both DMPA and NET-EN contain only one hormone—a progestogen. One disadvantage of these contraceptives, as perceived by the users, is that they can cause menstrual bleeding to become irregular and unpredictable or, occasionally, absent altogether. This side-effect, which is poorly understood, is common to all progestogen-only methods, including Norplant and the mini-pill.

There is no evidence that these menstrual disturbances have adverse health effects. In fact, the users of these methods experience on average less blood loss than with their normal menses and therefore are less exposed to the risk of anaemia. However, menstrual irregularity interferes with daily life and for sociocultural reasons is totally unacceptable in some settings. Thus, alternative injectables were developed which, like the combined pill, contain two hormones and induce a regular bleeding episode at monthly intervals. The trade-off is that these methods need to be administered monthly. The Programme, in collaboration with the pharmaceutical industry, has developed two such once-a-month injectables: Cyclofem (25 mg DMPA plus 5 mg estradiol cypionate) and Mesigyna (50 mg NET-EN plus 5 mg estradiol valerate). Both are very effective and, compared to DMPA or NET-EN, have the added advantage that fertility returns faster when their use is stopped. Both have been extensively tested in multinational clinical trials.

In 1991 the Programme undertook a major Phase III clinical trial in China to compare these two products with the widely used Chinese method, Injectable No. 1. The trial was completed in July 1991 and results will be reviewed at an investigators' meeting planned for 1992. Both Mesigyna and Cyclofem are also being compared in a Phase III clinical trial in Egypt with a view to evaluating possible introduction of either of these two injectable contraceptives in that country. This trial includes a study of the acceptability of these injectables among Egyptian women.

An introductory trial of Cyclofem has been completed in Mexico and similar trials are under way in Chile, Indonesia, Jamaica, Thailand, and Tunisia. Some 6000 women have now been recruited into these studies. Similar trials are planned in Colombia, Costa Rica, and Peru in 1992. The technology for the manufacture of Cyclofem has been developed and transferred to companies in Indonesia and Mexico, and it is hoped that the product will be registered in these countries in 1992.

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IUDs safe and effective at nine years of continuous use

Until recently, many of the copper-bearing intrauterine devices (IUDs) were approved for up to only four years of continuous use by national drug regulatory authorities as there was little information on the efficacy of these devices beyond four years. Long-term studies by the Programme on two copper IUDs—the TCu220C and TCu380A—have now provided data on nine years of use. The studies have included nearly 2800 women and the pregnancy rates for the TCu220C after seven and nine years of use were 4.9 and 5.4 per 100 woman-years, respectively. These rates represent an annual risk of accidental pregnancy with this device of approximately 1%. The TCu380A device has even lower pregnancy rates of 1.7 and 2.1 per 100 woman-years for seventh and ninth years of use, respectively, yielding an annual rate of less than 0.5%. Comparative trials of these devices are continuing and will provide information in the future on their safety and efficacy up to and beyond ten years of use.

Based on these studies, the United States Food and Drug Administration (USFDA) extended in August 1991 the approved duration of use of the TCu380A from six to eight years. This approval follows an earlier extension of the device's effectiveness claim from four to six years which was granted by the USFDA in 1989.

The IUD is one of the most commonly used methods of fertility regulation, especially in developing countries. It is estimated that there are more than 80 million IUD users with some 74 million in China alone.

The Programme has been actively involved in research on IUDs since 1974. This research has been focused on the long-term safety and efficacy of IUDs that are currently available and the development of new ones with lower rates of expulsion and fewer side-effects.

IUDs and pelvic inflammatory disease

In recent years the question has been raised, in developed countries in particular, as to whether the use of an IUD is related to pelvic inflammatory disease (PID) and whether long-term use is associated with severe forms of PID. In the past year, the large data base on IUDs accumulated by the Programme has been examined to clarify these issues. In a total of 22 908 insertions in 12 trials, it was found that the overall rate of PID was 1.6 cases per 1000 woman-years of use. Thus, a total of three cases of PID could be expected in two thousand women using an IUD in WHO studies in one year. This review of the data also showed that the risk of PID was seven times higher in the 20 days following insertion of the device, but thereafter the risk was low and remained constant for at least eight years of use. There was no evidence of an increase in the severity of PID with increasing duration of use.

New frameless IUDs

Two of the major side-effects of IUDs—pain and cramping, alone or together with bleeding—are thought to be caused by the difference in the size of the IUD and the size and shape of the uterine cavity. The Programme has been evaluating a new concept in IUDs in which the copper sleeves—which in currently available devices are placed on a plastic frame—are suspended from a nylon suture. The suture is inserted superficially into the uterine muscle at the time of insertion, leaving it and the copper sleeves to hang freely from the top of the uterine cavity. This device is presently being compared to the TCu380A IUD in a study involving 28 centres.

A number of studies have shown that IUD insertion immediately following removal of the placenta at delivery, or at any time from 24 hours to six

weeks after delivery, is associated with a high expulsion rate. This is probably due to a combination of the uterus returning rapidly to its usual non-pregnant size and the device being too small. The Programme will soon start pilot studies in up to six centres on two novel IUDs that are based on the general concept of the frameless IUD and have been designed specifically for insertion during the postpartum period.

Research on progestogen-induced endometrial bleeding

Unpredictable endometrial bleeding is a major side-effect of progestogen-only contraceptives—particularly the long-acting preparations—which greatly affects their acceptability. To address this problem the Programme is investigating the mechanisms of progestogen-induced bleeding at the endometrial level, evaluating different treatment modalities for progestogen-induced bleeding, and developing a methodology for the statistical analysis of menstrual bleeding patterns.

In 1991 the Programme initiated two research projects to investigate some of the mechanisms involved in endometrial bleeding. In one project, endometrial biopsies from Norplant users with regular and irregular bleeding patterns are being compared to those of women not using any contraceptive method with the aim of investigating structural and functional differences in the endometrium. Preliminary results from this study have already provided new insights into the vascular physiology of the endometrium. For instance, the study has found that during the normal menstrual cycle there are variations in angiogenic activity (formation of new blood vessels) and metabolism of a substance with vasoconstriction properties called endothelin. Moreover, data show that Norplant users and women not using contraceptives exhibit significant differences in endometrial capillary density and distribution of progesterone receptors. Differences have also been observed in endometrial endothelin content among Norplant users with irregular bleeding and those with amenorrhoea.

In the second project, two groups of Norplant users with regular and abnormal bleeding patterns are being compared. The latter group is being given an estradiol treatment via an Estraderm patch or a placebo, administered in a double-blind fashion. Endometrial estrogen and the distribution of progesterone receptors are assessed from biopsies taken on admission and after treatment.

As to the treatment of progestogen-induced endometrial bleeding—which is still a moot issue among scientists—a study has been initiated in six centres to compare the effects of: (*a*) a 14-day treatment with 50 µg ethinyl estradiol daily; (*b*) 2.5 mg estrone sulfate daily, and (*c*) a placebo. The women participating in the study are DMPA (depot-medroxyprogesterone acetate) users who experience a bleeding episode of more than seven days. A total of 1036 women were enrolled, and, of these, 275 women were randomized to one of the treatments for prolonged bleeding. Data collection will be completed in June 1992. Preliminary data indicate that, in the short term, ethinyl estradiol is effective in stopping irregular bleeding episodes in 92% of cases. The success rate achieved with estrone sulfate was not different from that observed with the placebo, being 72% and 73%, respectively.

With regard to the statistical analysis of bleeding patterns, a microcomputer software has been developed for the analysis of menstrual diaries which is based on the "reference period method" of analysis. This program, which includes the facility for displaying the results graphically, is being tested in several collaborating centres, and will be made available free of charge to interested scientists.

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Birth control vaccines: the progress continues

The WHO prototype vaccine had no adverse effects either on the pregnant animals or on the fetuses.

Research conducted by the Programme has led to the development of a prototype antifertility vaccine. This vaccine consists of a conjugate immunogen, formed from a synthetic fragment of the hCG (human chorionic gonadotrophin) molecule that is joined to the diphtheria toxoid as a carrier molecule, and an immunostimulant. These components are suspended in an emulsion vehicle for injection.

Between 1986 and 1988 the Programme conducted a clinical trial to assess its general safety (Phase I trial) in sterilized women. The next step—prior to efficacy studies—was to ensure that the vaccine would not produce any abnormalities in the fetus—should it fail to prevent pregnancy. Thus, during the biennium 1990–1991 teratology studies were conducted in rats and rabbits. (Anti-hCG vaccines do not prevent pregnancy in these species and this makes it possible to study the effects of such vaccines and their breakdown products in the body on fetal development). The results showed that the WHO prototype vaccine had no adverse effects either on the pregnant animals or on the fetuses. With the safety of the vaccine now quite well established, the Programme is planning to conduct clinical trials in 1992 to assess the effectiveness of the vaccine (the so-called Phase II trials).

Although studies done so far have shown that the prototype anti-hCG vaccine appears to be well tolerated and is apparently safe and immunogenic in humans, this version of the vaccine is unlikely to be suitable for wide-scale clinical use since it requires at least two injections, at an interval of several weeks, to elicit an anti-hCG immune response lasting for three to six months, depending on the individual. The Programme, therefore, has been developing an advanced prototype vaccine which is designed to elicit effective levels of immunity which will last for 12 months or more following a single injection. The advanced prototype anti-hCG vaccine consists of the same immunogen conjugate and immunostimulant used in the prototype version. But this time they are incorporated into a polymer designed to release the vaccine slowly over an extended but predetermined period of time. Although the Programme has opted for a vaccine capable of eliciting effective immunity for a period of 12–18 months following a single injection, vaccines of both shorter and longer durations of effect could be produced using the same technology.

Dose–response and toxicity studies are being carried out in rabbits and baboons to determine the optimal dose of the vaccine for generating the desired level and duration of immunity, and to see if this version of the vaccine is safe for testing in humans. Subject to a satisfactory outcome of these studies, a clinical trial to assess the safety of the advanced prototype anti-hCG vaccine could be initiated during the second half of 1992.

The advanced prototype anti-hCG vaccine may prove suitable for product development if it performs satisfactorily during the early clinical trials. However, it is rare in the course of drug development for a formulation tested in clinical trials to be the final version used for large-scale production. It is anticipated, therefore, that further modifications and improvements will need to be made to the vaccine as it progresses through the various stages of preclinical testing and clinical trials, and some studies have already been carried out with this need in mind. This research has included attempts to manipulate selectively the immune response using recently discovered molecules known to be, or suspected of being, involved in the natural regulation of immunity. Another approach has been the synthesis of alternative hCG fragments to increase the potency of the immunogen conjugate component of the vaccine while retaining its specificity for hCG.

Anti-trophoblast vaccines

The Programme's research on anti-trophoblast vaccines is aimed at the development of vaccines based on molecules which are found only in the membrane of the cells of the preimplantation embryo that would eventually form part of the placenta.

The traditional biochemical procedures used for the isolation of membrane components often lead to the alteration, or destruction, of molecules that are labile or present transiently and in low concentrations on the cell surface. This has caused major difficulties in identifying molecules on the surface of the preimplantation embryo which might represent suitable candidates for anti-trophoblast vaccine development. These technical problems have been largely overcome recently with the use of monoclonal antibodies (MAbs) and recombinant DNA technology which, when used in conjunction, permit the identification and isolation of molecules of interest. This research is being carried out as a multicentre collaborative programme involving the evaluation of MAbs brought to the Programme's attention by investigators working in the field, the generation of new MAbs in Programme-funded projects, the determination of the molecular structure of the more promising candidate antigens, and the preliminary in-vitro and in-vivo testing of the safety and efficacy of prototype vaccines based on these molecules.

Since this research was initiated in 1985, close to 15 000 MAbs have been tested, and current studies are focusing on nine of these MAbs which appear to be particularly promising. When used to immunize a small group of female baboons, the placenta-derived protein recognized by one of these MAbs was found to be capable of reducing their fertility in spite of the fact that only very small amounts of the protein were available for injection. Studies in 1991 focused on the biosynthesis of sufficient quantities of this protein to permit larger-scale efficacy studies to be carried out, as well as on the isolation and characterization of the molecules recognized by another four of these MAbs. All nine of these MAbs are currently being evaluated in a variety of experiments in an attempt to define the structure and function of the molecules with which they react.

An advanced prototype anti-hCG vaccine developed by the Programme may prove suitable for product development if it performs satisfactorily during the early clinical trials.

Update on vaginal rings

The development of vaginal rings has been one of the major research lines of the Programme since its inception. This research has led to the development of a ring which releases levonorgestrel continuously, at a rate of 20 µg per day. Extensive testing in 19 centres worldwide has confirmed its efficacy and acceptability. The main side-effects of this method are irregular menses observed in half of the users, a feature common to all progestogen-only methods, and also a risk of expulsion of the ring, mostly for older women and women of high parity. This ring is ready for production on an industrial scale and will be distributed by Roussel Laboratories Ltd. (UK). The company has applied for a product licence in the United Kingdom.

Other progestogen-only rings are also being studied by the Programme, such as the one designed for use by lactating mothers. This ring releases the natural hormone progesterone, which can enter breast milk from the circulation but is poorly absorbed orally, and thus does not affect the breast-fed infant.

One major advantage of the vaginal rings is that they are the only long-acting method which is under the control of the user—i.e., the woman can insert or remove the ring at will without help from a health care provider. Also, they release very small amounts of hormone at a constant rate, thereby reducing exposure to the hormone.

Antiprogestogens: from abortion to contraception

Studies in animals show that antiprogestogens can prevent pregnancy effectively. In humans, antiprogestogens could be given prior to ovulation as a contraceptive.

Antiprogestogens, as the name suggests, neutralize the action of the hormone progesterone. They do this by binding with high affinity to the progesterone receptor, consequently preventing the hormone from occupying its binding site on the receptor, which is a necessary step for it to produce an effect. Since, among other things, progesterone is essential for the establishment and maintenance of pregnancy, antiprogestogens have found their first use as agents for the termination of early pregnancy. Although several hundreds of such compounds have been synthesized to date, only one, mifepristone (RU486), has been studied extensively in humans. Mifepristone is also the only antiprogestogen that is presently registered for clinical use—in China and France since September 1988 and in the United Kingdom since July 1991. In all three countries the drug is licensed for use in medical termination of pregnancy with the recommendation that it be administered in conjunction with a uterotonic prostaglandin analogue.

Since without the action of progesterone the endometrium (the inner lining of the uterus) does not become biologically ripe for implantation of the fertilized egg (hence establishment of pregnancy), anti-progestogens could be administered just after ovulation to prevent implantation of the fertilized egg. Experiments in animals support this concept. In several species, including the mouse, rat, and monkey, treatment with an antiprogestogen shortly after mating has been shown to be effective in preventing pregnancy. In the rat, this antifertility effect was demonstrated in Programme-supported research to involve three mechanisms: (*a*) an alteration of tubal function resulting in accelerated transport and expulsion of embryos from the reproductive tract; (*b*) an arrest in the development of the preimplantation embryo; and (*c*) a decrease in the receptivity of the uterus. Likewise, in the human, mifepristone treatment given shortly after ovulation significantly affected endometrial development without disturbing the cyclic rhythm of menstruation. A study is under way in Sweden to determine if the alteration induced in the endometrium does indeed prevent the occurrence of pregnancy.

It is also well established now that in several species, including non-human primates and the human, antiprogestogens disrupt pituitary gonadotrophin secretion and, when given during the follicular phase of the cycle, arrest follicular development and ovulation. This has led to the speculation that antiprogestogens could be used in an estrogen-free type of sequential oral contraceptive pill. However, in most, if not all, of these studies mifepristone was given once or for a few days only in relatively high doses (in humans usually 100 mg or more) and no information is available on the mimimum daily dose needed to suppress ovulation reliably.

The Programme has initiated pilot studies in Santiago (Chile) to study the effects of continuous daily treatment with mifepristone on pituitary and ovarian function and on endometrial development. In women treated with 10 mg or 5 mg of mifepristone per day for 30 days, the cycles were longer than the pretreatment cycles by about 50% on average and ovulation was suppressed during treatment in all of them. Endometrial biopsy specimens showed a poorly developed or proliferative endometrium. In three of the five women given 5 mg of mifepristone the normal secretory activity of the endometrium was reduced, and in the other two endometrial secretions were completely absent. In contrast, in a third group of five women treated with 1 mg of mifepristone, only two had longer than normal cycles and ovulation was suppressed in only one of these two subjects. Disturbance of endometrial secretion patterns were found in three of the five biopsies; in the other two, the endometrium was inactive in one and proliferative in the other.

The findings in women taking 1 mg mifepristone daily—particularly that normal endometrial maturation was disturbed but the menstrual cycle was not prolonged—suggest that the antiprogestogen could be used as a new form of "mini-pill", provided a dose can be found that reliably disturbs endometrial maturation without significantly affecting the length of the cycle or ovarian steroid secretion. Recently published evidence in the guinea pig suggests that such a mini-pill might indeed be effective in preventing pregnancy. Obviously, detailed studies will be needed to ensure that continuous use of an antiprogestogen in low doses does not have adverse long-term effects.

An alternative approach, suggested by the results obtained in women given 5 mg or 10 mg mifepristone, would be to use the antiprogestogen in a sequential contraceptive pill regimen with a progestogen. Pilot studies supported by the Population Council and the Programme indicate that such a regimen allows the formation of a normal secretory endometrium and the occurrence of timely, well-controlled bleeding. In these studies, however, ovulation was not suppressed consistently in all treatment cycles, and hence further research is needed to establish a fully effective regimen.

Since mifepristone can block ovulation or retard endometrial development, depending on when the compound is given in relation to ovulation, antiprogestogens may have potential as postcoital contraceptives for emergency use. To explore this possibility, two randomized trials have been started in which efficacy and side-effects of a single dose of 600 mg mifepristone are being compared with those of the estrogen-progestin combination regimen which is used currently. Interim data from these two trials were published in 1991 and are most promising, since no clinical pregnancies occurred among the approximately 500 women given the antiprogestogen as compared to six pregnancies in women given the standard estrogen–progestogen treatment. Full data of these two trials are expected to be published in 1992.

Since mifepristone can block ovulation or retard endometrial development, it may be possible to develop antiprogestogens as postcoital contraceptives for emergency use.

Research on a vaccine against *Chlamydia*

The Programme, in collaboration with a private foundation, is funding research and development of a vaccine against *Chlamydia trachomatis*. The major protein in the outer membrane of the organism has been isolated and its chemical structure determined. The sites on the molecule where the antibody binds have been identified, and a prototype vaccine has been tested in an animal model for chlamydial pelvic infection. These studies suggest that vaccination with a preparation derived from molecular engineering can confer protection against chlamydial genital infection. Research has started on the vaccine delivery system as this will be all-important in ensuring that the optimal dose of the vaccine is delivered.

Chlamydial infection of the genital tract is probably the most common cause of tubal obstruction resulting in infertility. It is difficult to diagnose and treat and, in fact, often goes undiagnosed. Large-scale vaccination against the chlamydial organism offers the possibility of preventing or lessening the severity of pelvic chlamydial infection.

Injectables

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As with other contraceptive methods, efforts are under way to develop improved injectable formulations with fewer side-effects by lowering the overall steroid dose and by changing the formulation so as to avoid the high peak blood levels which follow the injection. This can be achieved with delivery systems such as microspheres which slowly release the steroid as they break down, or by modifying the size of the steroid crystals so that they act as a depot, slowly releasing the drug in the body. The Programme has chosen the latter, less expensive, approach to develop a new three-monthly injectable. The compound under study is an ester of the progestogen levonorgestrel, which is present in many oral contraceptives. However, it will take several more years of research before this preparation becomes available to family planning programmes.

Search continues for a simple, home-based method for predicting ovulation

A great deal of research effort by diagnostics companies and by agencies including the Programme has gone into finding a simple, accurate, robust, home-based method for predicting ovulation—as yet with little success.

Natural family planning (NFP)—i.e., methods of fertility regulation based on the observation of naturally occurring signs and symptoms of the fertile and infertile phases of the menstrual cycle—is used by a significant number of couples worldwide, at least at some stage of their fertile life. All of the commonly used methods of NFP require periodic abstinence, often quite long, from sexual intercourse. This makes the methods unattractive to many potential users as strong motivation is needed by couples to use NFP successfully for any length of time.

While it is fairly easy to determine the end of the fertile period in women, by detecting a rise in basal body temperature or by detecting predetermined amounts of the ovarian hormone progesterone in the urine, the accurate prediction of impending ovulation, the start of the fertile period, is far from straightforward. A great deal of research effort by diagnostics companies and by agencies including the Programme has gone into finding a simple, accurate, robust, home-based method for predicting ovulation—as yet with little success.

The most reliable indicator of approaching ovulation is probably a sustained increase in the levels of excretion products in urine of the hormone estradiol. A rise in the blood level of estradiol precedes ovulation by several days and therefore is attractive as a marker for the start of the fertile period. This increase is reflected by a similar rise in the excretion products in urine. Until recently there was no way to measure the most plentiful of these substances, estrone glucuronide, in urine other than in a laboratory. However, scientists in Melbourne, Australia, have developed a simple instrument that can be used in the home to measure estrone glucuronide in urine and also pregnanediol glucuronide, a product of progesterone, increasing levels of which mark the end of the fertile period.

In 1991, in collaboration with researchers in Australia, the Programme started a multicentre study of the home use of this instrument and the daily measurement of the ovarian hormones compared to the signs and symptoms used in conventional NFP methods. If the instrument proves to be accurate and easy to use, further development may be possible to make it suitable for general use. An advantage of this method is that both the start and the end of the fertile period are defined using the same method.

Another substance that may be a useful indicator of impending ovulation is the enzyme guaiacol peroxidase. The concentration of this enzyme in cervical mucus decreases as the level of estrogen rises prior to ovulation. Evaluation of the measurement of guaiacol peroxidase in cervico-vaginal fluid for definition of the start of the fertile period is being supported by the Programme in a multicentre study.

Symptothermal indicators of fertility unreliable for women nearing menopause

Determination of the fertile period in women approaching menopause is difficult owing to a variable proportion of non-ovulatory cycles in this population. The Programme has completed a study which examined the relation between ovarian hormone secretion and symptothermal markers of fertility in a group of 36 premenopausal women who contributed a total of 177 cycles of observation.

About 33% of the women experienced regular menstrual cycles that were potentially fertile, about 19% had cycles with no hormonal evidence of potential fertility, and the remainder had a mixture of both types of cycle. The conventional symptothermal indicators proved unreliable in distinguishing between the different types of cycle.