Vaccines for Fertility Regulation

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Summary

The research objectives of the Task Force on Vaccines for Fertility Regulation are to develop vaccines that will safely and effectively inhibit fertilization and vaccines that will safely and effectively disrupt implantation. As a result of funding constraints, the activities of the Task Force over the past eight years or so have focused on the development of a vaccine designed to neutralize the functions of the hormone, human choronic gonadotrophin (hCG), which is produced by the trophectoderm of the developing embryo and is essential for implantation and the maintenance of early pregnancy. Although there is a substantial amount of animal data on the efficacy of this novel approach to fertility regulation, there is a lack of relevant information on the long-term safety of such a method. The Task Force's mandate, therefore, was to produce an anti-hCG vaccine capable of eliciting an immune response specific to the intended target molecule in order to avoid cross-reactions that would lead to metabolic disturbances, the risk of immunopathology or other undesirable side effects, bearing in mind that this vaccine will be used by healthy and fertile women. In order to satisfy this requirement, the anti-hCG vaccine developed by the Task Force is based on a synthetic peptide representing the hCG-specific carboxyterminal region of the ß-subunit of the hormone (ß-hCG-CTP).

During the reporting period, the Task Force's prototype ß-hCG-CTP vaccine has been evaluated in a Phase I clinical trial. The principal objective of this study, involving previously electively sterilized women volunteers, was to assess the nature and intensity of any side effects associated with the vaccine's use in humans. Although the women taking part in this trial were not at risk of becoming pregnant, an estimate of the efficacy of the vaccine was obtained by measuring the level of anti-hCG antibodies that the vaccine elicited and then relating this to the amount of hCG to be neutralized in the maternal circulation at the peri-implantation stage of embryonic development. The results obtained in this trial have shown that the ß-hCG-CTP vaccine can elicit the production of antibodies to hCG and that the level of these antibodies appears to be high enough to confer an antifertility effect in fertile women. Moreover, no serious or unacceptable side effects were observed by the investigators nor reported by the trial volunteers. In view of these encouraging results, the Task Force is planning another clinical study, this time to evaluate the antifertility action of this prototype vaccine formulation in fertile women.

The duration of the immunity elicited by the prototype vaccine used in the Phase I clinical trial, several weeks to several months, is well short of the 12-24 months sought by the Task Force. In addition, the complex composition of the vaccine and the less than ideal nature of some of its constituents, would make this prototype unsuitable for wide-scale use. In parallel with the Phase I clinical study, therefore, work has continued on the development of improved and more acceptable anti-hCG vaccine formulations. The first phase of this work involves improving the current vaccine, notably by incorporating it into biocompatible and biodegradable slow-release delivery systems to extend its duration of action. Results obtained in these studies so far, indicate that high levels of anti-hCG

immunity, persisting for periods in excess of 12 months, can be elicited with such preparations in experimental animals. The second phase of this work is concerned with optimizing the anti-hCG vaccine to the point where it represents a safe, effective and acceptable pre-product formulation. Preliminary results indicate that the use of a combination of carefully selected and engineered hCG peptides greatly enhances the anti-hCG antibody response and that alternative carriers, adjuvants and delivery systems, offer promise in terms of producing an anti-hCG vaccine with enhanced efficacy, safety and a prolonged duration of activity following a single injection.

During the reporting period, the Task Force has implemented a new research programme to develop a vaccine directed against the trophoblast of the peri-implantation embryo. In contrast to earlier studies carried out in this area, in which classical biochemical approaches were used to isolate trophoblast membrane protein antigens of potential interest for vaccine development, the Task Force is employing monoclonal antibodies (MABs) and recently developed biotechnological procedures in order to identify, isolate, characterize and select relevant molecules more precisely. A sperm antigen classification system has also been initiated by the Task Force, using the same techniques and procedures as for the trophoblast antigen work, in an effort to standardize and rationalize the information being generated by the large number of investigators working in this area with support from other international and national funding agencies. This antigen classification project is considered by the majority of the investigators in the field to be an essential aid to vaccine development.

In preparation for these new activities, the Task Force carried out an international, multicentre collaborative project to evaluate and assess the large number of anti-trophoblast and anti-sperm MABs that were already available. A total of 111 MABs, 29 providing laboratories and 42 evaluating laboratories were involved in this project, the results of which were reviewed in a WHO-sponsored workshop held in conjunction with the sixth International Congress of Immunology in Toronto, Canada, in July 1986. As a result of these initial studies, a number of anti-trophoblast and anti-sperm MABs have been selected as reagents for the identification, isolation, characterization and selection of molecules for evaluation as components of prototype anti-trophoblast and anti-sperm vaccines.

The Task Force has continued to coordinate its research activities with other vaccine development programmes within WHO and with other international and national programmes engaged in the development of fertility regulating vaccines. This coordination has involved participation by representatives of other programmes and agencies in Steering Committee meetings of the Task Force and by Special Programme representatives in relevant meetings of other agencies.

THE RATIONALE FOR FERTILITY REGULATING VACCINE DEVELOPMENT

Although the number of fertility regulating methods currently available is probably greater now than at any time in the past, it is still not adequate to meet the widely varying cultural, religious, personal and service needs of all populations, particularly of those in the developing countries. Many of these methods act by exerting a pharmacological action at one or more points in the reproductive process, leading to the alteration or inhibition of a physiological function and resulting in an antifertility effect. This desired effect is often accompanied by less desirable side effects of varying types and intensity, which, together with the increasing concern being expressed about the sequelae of long-term use of many of these preparations, is having a major influence on their acceptability and continued use.

If vaccines could be developed which would safely and effectively inhibit fertility, without

producing unacceptable side effects, they would be an attractive addition to the present armamentarium of fertility regulating methods and would be likely to have a significant impact on family planning programmes. The theoretical advantages that a fertility regulating vaccine (FRV) would have over currently available methods of fertility regulation include: (a) lack of pharmacological activity and the often attendant side effects; (b) long-lasting action following only one or two injections; (c) administration by a procedure associated with positive health benefits; and (d) low manufacturing cost and ease of delivery within existing health services.

Essential to the development of FRVs is the identification of components of the reproductive system whose neutralization by immunological means will result in a safe, effective and acceptable antifertility effect, as well as the identification of appropriate animal models in which relevant preclinical studies of vaccine safety and efficacy can be carried out.

OPTIONS FOR FERTILITY REGULATING VACCINE DEVELOPMENT

Mammalian reproduction is a highly complex biological process involving diverse and specialized molecular systems and our knowledge of the number and type of molecules that are both specific to and essential for successful reproduction is rapidly increasing as the result of both clinical and basic research in the reproductive sciences. Immunization studies have demonstrated that many of these chemical entities, when suitably modified, are capable of eliciting an immune response which will neutralize the biological activity or destroy the structural integrity of the parent molecule, thereby reducing or inhibiting fertility in the immunized animal. Furthermore, there is a substantial body of information linking naturally occurring immunity to some of these molecules with certain types of infertility. These experiments of man and of nature indicate that there are many components of the reproductive tissues that could form the basis of antifertility immunogens for use in the development of FRVs.

Whilst virtually every step in the reproductive process is accessible to immune attack, not all represent attractive targets for FRV development. Immunization to some of these target molecules can result in a low level of antifertility efficacy and/or can produce unacceptable side effects, ranging from minor disturbances in endocrine function through to the more serious immunopathological sequelae of auto-immunity and immune-complex disease. In order to avoid these potential side effects and hazards, it is necessary to select carefully those immunogens that will produce safe, effective and acceptable antifertility effects. Ideally, such candidates for FRV development, would need to be: essential for the success of the reproductive process; accessible to immune attack; specific to the intended target and not represented in other body tissues or fluids; located in a site where a specific and controlled immune reaction will have no immunopathological or other undesirable consequences; and present only transiently or in small concentrations. These criteria are met by some molecules in the sperm membrane, the zona pellucida of the ovum, the trophoblast cell membrane of the peri-implantation blastocyst, and in the early placenta. In addition, some secreted products of these tissues, such as hCG from the trophoblast, also appear to be promising candidates. Prototype vaccines, incorporating natural and synthetic preparations based on several of these tissue-specific immunogens, have been produced and evaluated in animal and clinical studies.

CURRENT RESEARCH ON FERTILITY REGULATING VACCINES

There is currently a major interest in FRVs in many countries and several national and international agencies are funding work in this area. These studies cover virtually all aspects of basic research on the molecular events involved in gametogenesis, comparative evaluation of interactions between monoclonal antibodies, biosynthesis of antigens using recombinant DNA techniques, and clinical trials of prototype vaccine formulations.

The National Institute of Child Health and Human Development (NICHD) in Bethesda,

MD, USA, has recently iniated a programme of research in this area under its Reproductive Immunology Initiative. The projects supported by this programme are concerned primarily with the identification and characterization of gamete antigens which might represent suitable candidates for FRV development.

The National Institute of Immunology (NII) in New Delhi, India, has a large programme on FRV development and evaluation, ranging from the use of molecular genetics techniques to produce bioengineered vaccines directed at a variety of different reproductive targets, to the clinical evaluation of a number of different hCG vaccine formulations all of which use the whole ß-subunit of hCG as the primary immunogen (Talwar et al, 1986, 1987; Shaha et al, in press).

The Population Council in New York, NY,USA, is also supporting studies on the immunobiology of the gametes as well as a major programme of anti-hCG vaccine development in which the whole ß-subunit of hCG is used as the primary immunogen (Thau et al, 1985, 1987).

The USAID supported Contraceptive Development Programme (CONRAD) in Norfolk, VA, USA is primarily concerned with FRVs which will exert an effect prior to fertilization and is concentrating, therefore, on the identification and characterization of sperm antigens and antigens of the zona pellucida (Gupta et al, in press; Mahony et al, in press).

In addition to these major research programmes, there is a large number of investigators working independently in many countries in areas relevant to FRV development. Many of these activities have been stimulated by the recent expansion of knowledge of immune processes and developments in biotechnology and the increasing interest being shown by both academic and commercial institutions in the potential these developments offer for novel vaccine design and production.

The Task Force has defined research strategies and drawn up research plans in six principal areas. In addition to anti-sperm, anti-zona pellucida, anti-trophoblast and anti-hCG vaccines, studies have been proposed to reassess the feasibility of producing an effective local (secretory) immune response restricted to the lumen of the male or female reproductive tracts, and to carry out studies in the area of basic vaccinology relevant to the development of FRVs in general. Because of funding constraints, the Task Force's activities over the past biennium have been restricted to only two of these six areas of interest, namely the continuing development of anti-hCG vaccines and the initiation of preliminary studies aimed at the development of anti-trophoblast vaccines (Ada et al, 1986). However, some work has also been carried out, largely as a collaborative exercise with other agencies, on the establishment of a classification system for sperm membrane antigens.

Development of the anti-hCG vaccine

Virtually all vaccines in use and under development at the present time are directed against targets, such as bacteria and viruses, which express foreign antigens to which the vaccine recipient is not immunologically tolerant. Although hCG is a 'foreign' hormone, in the sense that it is produced in significant quantities only by the early embryo, the pregnant woman is tolerant to it and does not mount an immune response against it, either because hCG is very similar chemically to the endogenous pituitary hormone hLH or because of her prior exposure to hCG *in utero*. The development of an anti-hCG vaccine, therefore, is a totally new and experimental area of immunotherapy in which there is little previous information to guide investigators. To determine the feasibility of developing this novel approach to fertility regulation, two major questions concerning the efficacy and safety of the vaccine needed to be answered.

-- Could an anti-hCG vaccine break maternal tolerance to the hormone and elicit an immune response of sufficient magnitude to neutralize the hormone in the maternal circulation at the peri-implantation stage of 'gestation'?

-- Could the anti-hCG response so produced be restricted to the intended target so that cross-reactions with other normal body

constituents, particularly hLH, would not occur, and endocrine and metabolic disturbances and the potential risk of immunopathology avoided?

The mandate of the Task Force, therefore, was to develop a prototype anti-hCG vaccine to the point of initial clinical testing in order to obtain as much information as possible relevant to these two questions. Subject to a satisfactory outcome of the preclinical safety and efficacy work and the clinical study, further development would be justified to improve the vaccine to the point where it was suitable for use in family planning programmes. This final stage of development would be analagous to the structure/activity studies that are carried out with any new drug prototype in order to produce a preparation with maximum effectiveness and minimum side effects at the lowest possible dose.

The studies carried out under this section of Task Force activities address three principal objectives: the preliminary clinical evaluation of the safety and antifertility activity of the first generation anti-hCG vaccine; development of improved formulations of the current hCG vaccine preparation and the development of an optimized anti-hCG vaccine with improved components and characteristics; and the continued development of a baboon chorionic gonadotrophin (bCG) vaccine to permit the evaluation of the long-term safety and efficacy of this approach to fertility regulation in a relevant animal model system.

Phase I clinical evaluation of the first generation anti-hCG vaccine

The first generation anti-hCG vaccine developed by the Task Force, consists of a synthetic peptide representing the ß-hCG carboxyterminal 109-145 peptide (ß-hCG-CTP) coupled to a diphtheria toxoid (DT) carrier molecule, mixed with a muramyl dipeptide (MDP) adjuvant, and suspended in a squalene-arlacel-saline emulsion vehicle (Griffin, 1985, 1986; Stevens, 1986a-c). This complex preparation

Table 1. Composition of the vaccine and placebo preparations administered in the five dosage groups in the Phase I clinical study of the anti-hCG vaccine

Group number	Vaccine recipients	Placebo recipients	Immunogen (µg)	Adjuvant (µg)	Vehicle (µl)
I	4		50	25	25
		2		25	25
II	4		100	50	50
		2		50	50
III	4		200	100	100
		2		100	100
IV	4		500	250	250
		2		250	250
V	4		1000	500	500
		2		500	500

was formulated immediately prior to injection by dissolving the peptide-carrier conjugate and MDP in isotonic saline and subsequently mixing this aqueous component with the squalene oil, using arlacel as the emulsifying agent. Vigorous mixing of these components is required in order to generate the high shear forces needed to achieve satisfactory emulsification.

Although no adverse side effects had been detected in the extensive preclinical toxicity, safety and efficacy studies carried out with this vaccine formulation in mice, rats, rabbits and baboons, no information had been obtained on the tolerance to, and immunogenicity of, this formulation in humans. Therefore, the principal objective of this Phase I clinical trial was to determine what, if any, side effects were produced by this vaccine. In addition, the levels of anti-hCG antibodies elicited by the vaccine were measured and compared to those that would be expected to confer antifertility efficacy in fertile women.

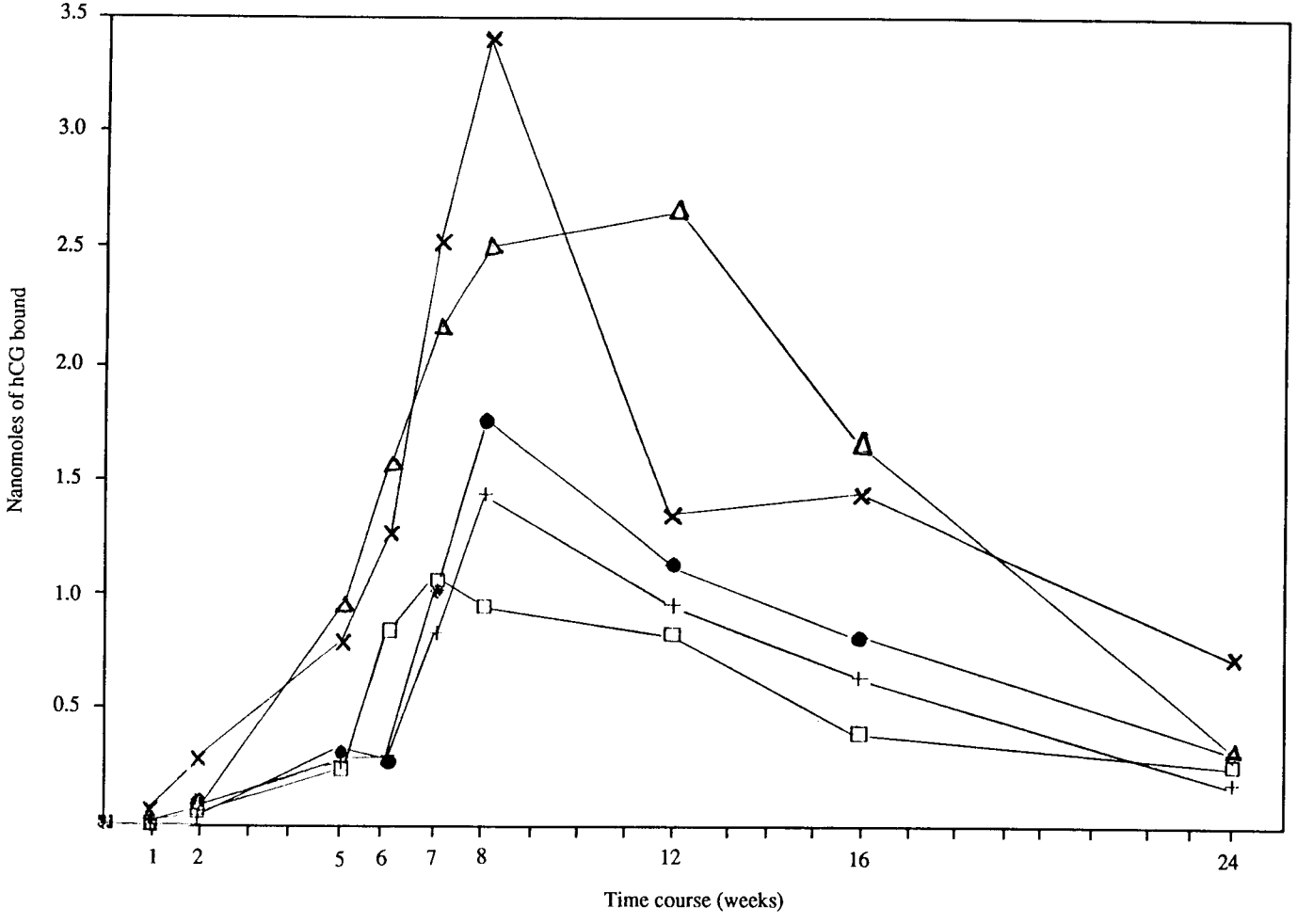
A total of 30 previously electively sterilized women volunteers were needed for the trial and recruitment was initiated in November 1985, following receipt of the necessary WHO, institutional and government approvals (Jones, 1986a-b). Telephone inquiries, requesting additional information about the trial, were received from a total of 185 potential volunteers of whom 93 were considered suitable candidates. Of these, 61 expressed an interest in taking part in the trial and 47 were subsequently selected for screening. A total of 13 women were excluded at the screening stage, for a variety of social, psychological and medical reasons, leaving the required 30 with four replacements, should these be needed.

In the interests of safety, necessitated by the novelty of the vaccine and the lack of previous clinical information with this type of formulation, the trial was conducted in a modified "dose-finding" manner in one centre. Six of the 30 women volunteers were assigned to each of the five dose groups indicated in Table 1, the highest of which, Group V, being the dose expected to elicit a level of immunity sufficient to confer antifertility efficacy, based on the results obtained in the baboon efficacy studies. Of the six women in each dose group, four received the complete vaccine and two received a "placebo" preparation consisting of the MDP adjuvant and emulsion vehicle only. Immunization was effected by two injections into the gluteal muscles at an interval of six weeks and the subjects were followed up on an in-patient and out-patient basis for a total of six months. A large number of routine and trial-specific examinations and laboratory investigations were carried out over this period.

The majority of vaccine recipients in all dose groups produced antibodies to hCG as well as to the DT carrier component of the vaccine. The anti-hCG antibody levels in Groups I, II and III exhibited only small dose-dependent increments whereas those in Groups IV and V did not follow this pattern and were much lower than Groups I-III. The only side effects considered significant by the resident physician, were transient muscle and joint pains reported by a few subjects. These symptoms, believed to be produced by the MDP component of the vaccine, were most marked in subjects in Groups IV and V but were satisfactorily controlled with analgesics and did not cause any of the volunteers to withdraw from the trial. Recovery in all cases was complete and there were no associated relevant serological abnormalities. Two subjects withdrew from the study after the first injection for reasons unrelated to the trial. A third subject was excluded after she converted to DT skin test positive following the first injection of the vaccine.

In view of these unexpected results, a close inspection was made of the patient records and laboratory data and a positive correlation was found between the intensity of the reported side effects and poor anti-hCG antibody responses in some of the vaccine recipients. In all cases, these phenomena were associated with apparent or suspected instability of the emulsion vehicle. Because of the difficulty of developing, manually, the high shear forces needed to generate adequate emulsification, the vehicles of some of the vaccine doses formulated were probably unstable and separated into their oil and water phases soon after injection. Thus, instead of the

Fig. 1. Magnitude and duration of anti-hCG antibody titres raised in the five hCG vaccine dose groups in the Phase I clinical trial



Key: □ : Group I; +: Group II; ●: Group III; Δ : Group IV; x: Group V.

desired slow and prolonged release of vaccine, it was likely that a rapid and transient release had occurred, producing the reported myalgia and arthralgia and the poor anti-hCG antibody responses.

It was decided, therefore, to form two additional treatment groups, Groups IVa and Va, of eight more volunteers who would receive the same vaccine doses as the subjects in Groups IV and V but this time in an emulsion vehicle which had been made more stable by minor changes to the relative proportions of its constituents. This plan was approved by the appropriate drug regulatory committees and authorities, the subjects recruited and the immunizations carried out. The incidence and severity of the myalgia and arthralgia reported by the subjects in Groups IVa and Va were significantly less than those reported by their predecessors in Groups IV and V and were most evident in Group Va. These observations would suggest that the amount of MDP to be used, in this particular vaccine formulation, should be limited to the Group IV/IVa dose of 250 μg or less. The anti-hCG antibody responses in Groups IVa and Va were considerably improved and formed with the Groups I, II and III data, a clear dose-response pattern (Fig. 1), both in terms of antibody titres and, to a lesser extent, duration of antibody production.

Because of known species differences in the structures of baboon and human chorionic gonadotrophins and the time-course and mechanism of implantation in these two species, it is difficult to determine, from previous baboon antifertility studies, what levels of anti-hCG antibody will confer antifertility efficacy in the human. However, on the basis of the concentration of hCG in the maternal circulation at the time of implantation, it is estimated that an antibody level of approximately 0.5 nanomoles of hCG binding capacity would prevent implantation and thereby confer antifertility efficacy in women. As can be seen in Figure 1, this titre threshold has been exceeded in all of the five dose groups receiving the complete vaccine.

As part of the intensive analysis of the serum samples obtained in the course of this Phase I trial, immunofluorescent staining studies have been carried out to determine the incidence and nature of any tissue cross-reactivity exhibited by these sera (Burek et al, 1986a; Rose et al, 1988). A total of four serum samples were obtained from each of the 30 volunteers, in weeks 5, 7 and 26 after injection of the vaccine, with a pre-immunization sample serving as an individual-specific control in each case. These sera have been tested against a panel of rat, baboon and human tissues prepared as fresh cryostat sections (Burek et al, 1986b).

Virtually all sera exhibited reactivity against rat cardiac, skeletal and smooth muscle. However, the frequency and intensity of these reactions were essentially the same in the pre-immune sera as in the post-immunization samples and these results were not considered to be clinically significant.

Weak reactions against baboon pancreas were observed with samples from four individuals. These reactions were transient, appearing in samples taken five weeks after injection of the vaccine and disappearing by week 26. A closer examination of the stained sections indicated that the antisera were binding to somatostatin secreting cells in the pancreatic islets. However, this reactivity could not be absorbed out using purified somatostatin, in either the natural or straight-chain forms. Although no signs of pancreatic dysfunction and damage were seen in the preclinical baboon efficacy and safety studies, nor detected in the detailed histopathological examinations that were carried out on these animals postmortem, further studies are underway to identify the antigen involved and the long-term sequelae of this reaction.

Two of the 30 sets of sera exhibited binding with human pituitary sections. In the first case this activity was present only in the week 26 serum sample and not in any of the other samples from this individual; in the second case the reactivity was observed in all of the samples with the most intense reaction being exhibited by the pre-immune serum. A closer examination of the stained tissue indicated that binding was not specific to any one cell type and appeared to be randomly distributed and restricted to the

intracellular compartment. In view of the difficulties involved in obtaining fresh human tissues for definitive immunofluorescence studies, and the known artefacts that can occur as the result of non-specific reactions and postmortem changes in the tissue substrates, these results are not considered significant. However, studies are underway to characterize further the reactions observed, to identify the cell types and the antigen involved, and to determine the long-term sequelae of this apparent antibody binding.

With the successful completion of this first clinical trial of a synthetic peptide based antifertility vaccine, it is important to review the objectives of the trial, how well these objectives have been met, and the other information that has been obtained during the course of this study.

The results obtained in the Phase I clinical trial indicate that the Task Force's primary objective has been successfully achieved, in that the desired type of immunity has been elicited in most of the vaccine recipients without the production of unacceptable side effects. Furthermore, the antibody levels attained in all responders are estimated to be in excess of those expected to confer antifertility efficacy.

In view of these encouraging results, the Task Force is proposing to carry out a limited study with the current ß-hCG-CTP vaccine formulation, in a small number of fertile women volunteers, to determine if anti-hCG antibody levels in excess of 0.5 nanomoles of hCG binding capacity will provide an antifertility effect. However, before seeking approval to conduct this study in women it will be necessary to carry out further animal experiments in order to determine if any fetal abnormalities are associated with the vaccine's use. This will involve both an assessment of "chemical teratology" in rodents and rabbits as well as a concurrent assessment of "immunoteratology" in baboons or another relevant primate model. The latter study is particularly difficult to carry out in view of the paucity of background information in this area and the need to use a large number of animals in order to obtain statistically significant data. Although negative data will not be conclusive, the Task Force considers it important to attempt to obtain as much information as possible before conducting an efficacy evaluation of the current vaccine formulation in women. Protocols for these animal experiments and for the clinical study have been drafted in consultation with Task Force scientists and clinicians and with representatives of the pharmaceutical industry and national drug regulatory authorities. A further supply of the ß-hCG-CTP vaccine, needed for these additional animal and clinical studies, is currently being prepared, under the appropriate GMP conditions.

Valuable additional information has been obtained in the Phase I clinical trial, relevant to the development of a safe, effective and acceptable anti-hCG vaccine suitable for wide-scale application, particularly in the developing countries. This important information concerns all components of the current ß-hCG-peptide prototype vaccine formulation.

HCG Peptide: The 109-145 ß-hCG-peptide used in the current vaccine appears to be capable of eliciting antibody levels estimated to be several fold higher than that needed to confer antifertility efficacy. However, future vaccine production would be greatly facilitated if one or more short peptides of ß-hCG could be identified which retained the ability to elicit high levels of specific immunity to hCG and which were cheaper and easier to manufacture and characterize than the long peptide in the current formulation.

DT carrier: Out of a total of approximately 80 injections with the ß-hCG-CTP vaccine that were made during the course of the trial, adverse reactions to the DT component were seen in two instances. Whilst this may appear to be a very low incidence, it would probably be regarded as an unacceptably high level when large-scale use of the vaccine is considered.

Adjuvant: Comparison of the clinical trial data with those derived in the preclinical animal studies would suggest that the human dose of the MDP adjuvant need not exceed 200-250 µg in order to elicit adequate anti-hCG immunity. Limiting the MDP dose to this level would greatly reduce, and perhaps even eliminate, the

occassional and transient arthralgia and myalgia reported by some of the trial volunteers in the high-dose groups.

Vehicle: The complex and highly viscous emulsion used as the vehicle for the current vaccine is obviously not suitable for use beyond the preliminary stages of clinical testing. Although it may prove possible, by mechanical means, to produce a stable emulsion in a reliable and repeatable manner, the short duration of the immune response elicited by the ß-hCG-CTP vaccine delivered in this vehicle, necessitating frequent booster injections, would argue against its use in further vaccine development.

These findings justify the decision taken by the Task Force to evaluate a number of alternative formulations, as well as additional immunogens, immunostimulants and vaccine delivery systems, in order to produce improved vaccine preparations that will be suitable for eventual product development.

Development of improved and optimized anti-hCG vaccines

This work is being carried out in two phases. The first phase involves an evaluation of alternative delivery systems for the existing ß-hCG-CTP:DT immunogen and MDP adjuvant in order to find a replacement for the squalene-arlacel-water emulsion. In this area, the Task Force has continued with its studies on microsphere delivery systems, prepared with biocompatible and biodegradable polymers. The advantages of this particular approach to vaccine delivery are that microspheres can be individually engineered to meet the varied physico-chemical requirements of the different vaccine components, thus permitting the sustained release of the encapsulated vaccine over long periods of time and at predetermined release rates. It would be possible, therefore, to prepare vaccine formulations with pre-set durations of action ranging from a few months to several years. Furthermore, these preparations appear to be capable of eliciting high levels of anti-hCG immunity of long duration following a single injection of vaccine. Last but not least, this approach offers the added advantage of long-term vaccine stability under a wide range of climatic and storage conditions.

During the reporting period, a large number of microspheres, consisting of different ratios of polylactic and polyglycolic acid, have been prepared and evaluated in terms of their ability to elicit anti-hCG antibody titres of the required magnitude and duration. Four of these formulations produced titres which persisted, for at least six months, at levels in excess of those produced by the emulsion delivery system, and additional studies with the more promising of these formulations have indicated that anti-hCG antibodies can be elicited for a period in excess of twelve months. Additional supplies of the more promising preparations have been produced in larger quantities for further studies and a number of new formulations are also being prepared for comparative evaluations.

The second phase of this work is concerned with optimizing the anti-hCG vaccine to the point where it represents a safe, effective and acceptable pre-product formulation suitable for large-scale clinical trials. The studies being carried out in this area involve optimization of all components of the vaccine.

HCG peptide: The ß-hCG-CTP component of the immunogen in the current vaccine formulation, consists of a synthetic peptide 37 amino acids long. In spite of recent advances in chemical and biological peptide production methods, the preparation of a peptide of this length is still an expensive and difficult undertaking if the product is to meet the stringent specifications demanded by drug regulatory authorities. The Task Force has been conducting studies, therefore, to identify and subsequently evaluate a variety of additional peptides representing both sequential and conformational immunogenic determinants (epitopes) on the ß-hCG molecule (Stevens, 1986d).

The following summary of the studies carried out in this area over the reporting period, illustrates the complexity of this work and the potential offered by this approach for the development

of totally novel peptide immunogens, selected and engineered to provide enhanced immunogenicity whilst retaining specificity for hCG.

In natural ß-hCG, the amino acid residues forming the 38-57 region of the molecule exist as a loop structure formed by a disulfide linkage between the cysteine residues at each end of this peptide. Since previous studies with other molecules have demonstrated that such loop structures are potent immunogens, perhaps because of their prominent surface presentation on the molecule, a 38-57 loop peptide was synthesized and evaluated in rabbits. Although this loop peptide proved to be highly immunogenic, the antibodies raised to it cross-reacted with hLH. Further studies were carried out, therefore, with a number of intermediates of both straight chain and loop versions of the 38-57 peptide in order to develop an analogue capable of eliciting high levels of antibodies devoid of hLH cross-reactivity.

Comparative mapping studies have identified the 43-50 sequence as the hCG-specific epitope within the 38-57 peptide. An analogue of this epitope, consisting of the 43-50 straight chain peptide linked at its amino-terminal residue to a hexa-proline spacer, proved to be more immunogenic in generating hCG-specific antibodies than the 43-50 peptide alone but not as immunogenic as the 38-57 loop peptide.

Complementary studies were carried out to define the regions within the 38-57 loop peptide that were responsible for eliciting antibodies crossreacting with hLH. These activities were found to reside in the regions, 30-42, 45-57 and 50-62 of the molecule. On the basis of the information gained from these studies, three experimental loop peptides have been prepared with amino acid substitutions designed to remove the hLH crossreactivity and to confer some degree of α helical structure on the peptide. One of these"helical" peptides has been found to be more immunogenic than its straight chain counterpart, and whilst it is not as immunogenic as the native 38-57 loop, it does produce antibodies specific for hCG. When this peptide is given, as a combined immunogen with the 109-145 ß-hCG-CTP, antibodies are elicited at levels much higher than those produced by either of the peptides alone. In addition, other surface epitopes on the beta-hCG molecule have been identified using predictive rules for molecular structure or by the binding properties of ß-hCG specific monoclonal antibodies. Peptides representing some of these epitopes have been synthesized and evaluated and a number of regions on the ß-hCG molecule have been identified which could form the basis for the development of new anti-hCG immunogens. These data indicate that immunogens consisting of a number of short and novel peptides, selected on the basis of their immunogenicity and hCG specificity, could form the basis of a prototype second generation anti-hCG vaccine meeting the Task Force's requirements.

New carriers: The carrier molecule used in the first generation anti-hCG vaccine is a purified form of clinical grade diphtheria toxoid (DT). Although this DT is a single molecular weight fraction, it is still a chemically ill-defined and heterogeneous preparation. Furthermore, the repeated use of a vaccine incorporating this material produces a low but significant incidence of delayed type hypersensitivity (DTH) reactions. As stated earlier, such DTH reactions were observed in two out of 30 women receiving the complete vaccine formulation in the Phase I trial. Although this incidence of DTH reactions presents no difficulties in the context of a small-scale clinical trial, it is almost certainly too high to permit a DT-containing vaccine to be considered for wide-scale clinical use.

The Task Force has continued, therefore, with its search for clinically acceptable molecules to evaluate as alternative carriers for the hCG peptide immunogens. In studies carried out with ß-hCG peptides conjugated to a purified protein derivative (PPD), obtained originally from *Mycobacterium tuberculosis*, good anti-hCG antibody responses were elicited and the frequency and intensity of DTH reactions were found to be much lower than those produced by conjugates containing DT. Further studies are planned with single molecular weight species of *M. leprae* PPD obtained by recombinant DNA procedures.

The principal advantage of these materials is that their chemical structures are known. However, they do have a major disadvantage in that they will not confer protection against tuberculosis but will render recipients skin test positive on subsequent challenge. The clinical and epidemiological implications of this "false positivity" would need to be carefully discussed before embarking on a major research programme to develop an anti-hCG vaccine, or any other vaccine, incorporating these materials as the carrier component of the immunogen.

New adjuvants: Although the MDP analogue in the current vaccine formulation has been approved for use in clinical trials, it may not be suitable for inclusion in a vaccine for wide-scale clinical use except at lower doses which may not confer immunity of sufficient magnitude or length in all recipients. There are a large number of additional MDP analogues and other experimental immunostimulants available, but the amount of immunological, pharmacological and toxicological information on these compounds is limited and is not sufficient to permit the selection of an alternative preparation suitable for clinical trials. The Task Force is continuing, therefore, with its studies to compare the properties of alternative immunostimulants that might be suitable for clinical use.

A number of thiol compounds have been tested during the reporting period in an effort to identify a clinically acceptable alternative to MDP, but none was found to be suitable for this purpose. Although no further studies are planned with these particular compounds, these studies did allow the confirmation of earlier findings that an adjuvant may be needed only in the first injection for a vaccine to elicit an adequate immune response.

New delivery systems: As indicated earlier in this chapter, the vehicle used in the prototype hCG vaccine is a high-viscosity water-in-oil emulsion which requires careful preparation immediately prior to injection. Although it is possible that the *in vitro* and *in vivo* instability problems encountered in the Phase I clinical trial might be overcome by preparing the emulsion using a machine capable of achieving high shear forces, it is unlikely that a vaccine for widespread clinical application will be administered in this form in view of the limited duration of the immune response elicited by preparations administered in this vehicle. The Task Force has continued, therefore, with its studies to develop and evaluate alternative delivery systems capable of producing an effective level of anti-hCG immunity of long duration, preferably following a single injection of vaccine. In addition to the work with copolymer microspheres referred to earlier, the Task Force has also carried out experiments in rabbits with a number of liposome and iscom (immunostimulating complexes) preparations, incorporating various combinations of the ß-hCG-CTP:DT conjugate and MDP adjuvant.

Both of these vaccine delivery systems appear to be well tolerated by the experimental animals and elicit substantial levels of anti-hCG immunity. However, in terms of duration of effect, neither liposomes nor iscoms proved to be as effective as the microspheres.

Development of a baboon CG vaccine

Although the primate chorionic gonadotrophins appear to have the same physiological functions, there are substantial species differences in the chemical structures of these hormones and in their secretion profiles throughout gestation. As a result of these species differences, antibodies raised in baboons to the heterologous ß-hCG-CTP vaccine cross-react with endogenous baboon CG (bCG) by approximately 5% compared to hCG. Whilst this comparatively low level of crossreaction is sufficient to produce an antifertility effect in the immunized baboons, it may not be sufficient to stimulate, either qualitatively or quantitatively, all of the acute or chronic side effects that might occur when the homologous ß-hCG-CTP vaccine preparation is used in women.

The Task Force has continued, therefore, with its programme to develop a baboon model system in which a vaccine based on the animal's own CG can be used to evaluate extensively these safety issues. These studies have proved

technically difficult and costly and have not yet been satisfactorily completed. If solutions can be found to the technical problems encountered, it may prove possible to develop a ß-bCG-CTP vaccine that is equivalent to the ß-hCG-CTP preparation. This anti-bCG vaccine would enable more meaningful data to be generated in baboons concerning the safety of this novel approach to fertility regulation.

The first step in the development of a ß-bCG-CTP vaccine is the elucidation of the amino acid sequence of the baboon hormone in order that the appropriate peptide can be synthesized. These studies have followed two separate but complementary approaches.

In the first set of studies, bCG is isolated from baboon pregnancy urine and purified for subsequent classical protein sequence analysis. Major unforeseen problems and delays have been encountered in this programme, resulting from the transient nature of the secretion of the baboon hormone, its inherent lability, and the difficulties of collecting sufficient quantities of baboon pregnancy urine in an uncontaminated form. Improvements have been made continuously by Task Force scientists in the procedures used for the collection of baboon pregnancy urine and its subsequent processing to obtain urinary bCG. Recently, several milligrams of purified bCG have been prepared with a biological activity corresponding to 10,000 IU of hCG per mg from which the ß subunit has been isolated for amino acid sequence analysis. In accordance with established protein sequencing procedures, the ß-bCG has been subjected to enzymatic digestion in order to obtain fragments of the molecule that can be analyzed using automated equipment. These fragments have been purified and separated by reverse-phase HPLC to yield 6-8 fractions with different physicochemical properties reflecting their location in the ß-bCG molecule. Preliminary amino acid analyses on these fractions are currently underway and it is anticipated that a tentative structure for part or all of this molecule might be available early in 1988.

In the second set of studies, recombinant DNA techniques have been used to determine the nucleotide sequence of the bCG gene, from which the amino acid sequence of the hormone can be deduced. These studies have identified several genes coding for CG in the baboon placenta, and it is not clear how many of these genes there are, how closely related they are to each other, or which one is responsible for producing the physiologically and immunologically important form of bCG. In an attempt to resolve these issues, the Task Force has carried out studies to assess the biopotency and immunological reactivity of heterodimers, consisting of the α subunit of bovine LH and the alleged ß subunit of bCG, produced using a mammalian cell expression system. The gonadotrophic potencies of the expressed products have been determined using the Leydig cell bioassay, and their immunological reactivities have been assessed in radioimmunoassay (RIA) using monoclonal and polyclonal antisera to ß-hCG-CTP and the proposed baboon counterpart. To verify the authenticity of the expressed materials, autoradiographic studies have been carried out to determine their presence and location in human and baboon placentae.

None of the expressed heterodimer products obtained so far has exhibited the appropriate activities in all of these systems. From the available data, it is not clear if the expressed material is a gonadotrophin or another, irrelevant, gene product. Comparative studies are underway to determine if one of the other bCG genes in the baboon placenta codes for a more relevant material than that already studied, and further RIA studies are also underway to determine the tissue specificity and localization of the putative antigenic material.

It is anticipated that a definitive answer to the bCG sequence question will be provided from the purified baboon pregnancy urine material (Bambra, 1987), either directly by total amino acid sequence analysis, or indirectly by using partial amino acid sequence data to prepare oligonucleotide probes for screening gene libraries prepared from baboon placentae at the peak of bCG secretion. The relative molecular sizes of the naturally occurring and expressed materials will be compared using electrophoresis, immunoblot procedures and autoradiography.

From the data generated in these two complementary research activities, it should prove possible to detect and characterize the C-terminal region of ß-bCG and to synthesize the appropriate peptide for formulation into a ß-bCG-CTP vaccine.

Once a relevant ß-bCG-CTP vaccine has been prepared, meaningful studies can be carried out in the baboon to assess the efficacy and safety of this homologous preparation. A successful outcome to these studies will provide greater confidence for proceeding to a clinical evaluation of the antifertility efficacy of the the current ß-hCG-CTP vaccine as well as for the development of improved anti-hCG vaccine formulations suitable for wide-scale clinical application.

Development of an anti-trophoblast vaccine

At its meeting in September 1985, the Special Programme's Scientific and Technical Advisory Group (STAG) recommended that the Task Force should expand its activities to include studies on additional targets for FRV development. In order not to duplicate the work of other agencies in this area, which is largely restricted to the development of anti-gamete vaccines, the Task Force has initiated studies on a vaccine based on membrane antigens of the early trophoblast. Although there are many investigators working on the immunobiology of the early trophoblast, this is largely in terms of its role in preventing immunological rejection of the "fetal allograft", and the Task Force studies are the only international, multidisciplinary collaborative vaccine development programme in this area.

Previous studies carried out, both by Task Force scientists and other investigators, had focused on the identification of membrane expressed molecules that could be isolated from the trophoblast using classical mechanical and biochemical extraction procedures (Johnson, 1985; Stern et al, 1987). The comparative harshness of this approach can result in the partial or complete destruction of the more labile membrane components, many of which may be molecules of interest for vaccine development. The Task Force has decided, therefore, to use a combination of monoclonal antibodies (MABs) and molecular genetics techniques to identify, isolate and characterize trophoblast membrane antigens that might represent suitable candidates for development into anti-trophoblast vaccines.

The research strategy being employed by the Task Force in this area involves the use of MABs that satisfy the following three criteria:

-- tissue-specificity for human trophoblast;

-- cross-reactivity with similar or equivalent tissues in other mammalian species;

-- ability to disrupt or inhibit the function of trophoblast *in vitro* and/or *in vivo*.

By establishing these three criteria at the outset of its studies, the Task Force is aiming to develop anti-trophoblast vaccines which will not produce cross-reactions to other non-target tissues; which can be evaluated for safety and efficacy in a relevant animal model; and which will be directed against antigens expressed on the cell surface, and which are accessible, therefore, to antibodies and immune cells in the maternal circulation. MABs satisfying these three criteria can then be used to isolate antigens expressed on the surface of the trophoblast membrane and to screen the products of expression systems in which genes coding for trophoblast antigens have been inserted. The data generated in these studies will permit a range of synthetic peptide immunogens to be prepared for comparative evaluations of their potencies as anti-trophoblast vaccine components.

As an initial step in this new research programme, the Task Force has carried out a systematic evaluation of a large number of anti-trophoblast monoclonal antibodies that had already been produced by investigators in the field. The data generated in these studies were reviewed and discussed at a workshop (Anderson et al, 1987), jointly organized by WHO and Family Health International (FHI), and held in Toronto, Canada, in June 1986 in conjunction with the third International

Congress on Reproductive Immunology and the sixth International Congress of Immunology. A total of 44 such antibodies alleged to satisfy the primary criterion of trophoblast specificity were obtained from 15 investigators and further characterized, in coded form, in terms of the types of tissue with which they reacted, the location of these reactions within the tissue, and the nature of the putative antigenic material. The results obtained in these preliminary studies are summarized in Table 2.

Table 2. Tissue reactivities of anti-trophoblast monoclonal antibodies

Tissue reactivity observed	Number of monoclonal antibodies
First trimester and term human placenta	19
Term human placenta only	6
Extensive cross-reactivities with other human tissues	11
Non-reactive with first trimester and term human placenta	8

Species cross-reactivity was assessed against baboon, marmoset, donkey, horse, cow, pig and rodent placentae. Five of the 44 MABs exhibited tissue-specific cross-reactivity with baboon placental tissue and many exhibited a variable degree of cross-reactivity with rodent placental or embryonic tissues. However, the data generated with rodent tissues showed poor correlation between investigating laboratories.

Tissue location and antigen characterization studies were carried out on detergent-solubilized isolated human placental syncytiotrophoblast plasma membranes using immunoblot and radioimmunoprecipitation techniques. In the immunoblot studies, five of the 44 MABs reacted with clearly defined bands of solubilized material with molecular weights of approximately 115 kDa, and 14 MABs identified protein antigens in the radioimmunoprecipitation studies. Further immunohistological studies and enzyme capture assays indicated that four out of the five MABS that reacted with solubilized material in the immunoblot experiments were directed against heat-stable placental-like alkaline phosphatase (PLAP) and an additional four MABs appeared to be directed against the cell surface receptor for transferrin. However, five other MABs appeared to react to novel solubilized components of placental syncytiotrophoblast plasma membrane and two of these MABs also reacted with baboon placental tissue. These two MABs appeared to be directed against two glycoprotein antigens with molecular weights of 43 kDa and 76 kDa, respectively.

These preliminary studies have identified, therefore, at least two and perhaps as many as five MABs, out of the original group of 44, that appear to satisfy two of the three criteria established by the Task Force. Further studies are underway and planned with these high priority reagents in order to isolate and characterize the trophoblast membrane protein antigens with which they react as a prelude to the synthesis of these materials for evaluation in prototype anti-trophoblast vaccines. The Task Force is maintaining contacts with investigators working in this field and further MABs, meeting the same stringent requirements, will be added to this panel of reagents as they become available.

Progress has been made also in the recombinant DNA project which is being carried out to complement the MAB studies. A human placental gene library has been established and vectors containing cDNA coding for human placental proteins have been inserted into cloned mammalian host cells. The high priority MABs will be used to screen these host cells for the presence of relevant human placental gene products expressed on their plasma membranes. Clones exhibiting the desired expression products will be expanded to obtain sufficient quantities of genetic material, or expressed protein, for the sequence analyses needed prior to the synthesis of peptides for subsequent evaluation as candidate immunogens for anti-trophoblast vaccines.

Table 3. Tissue reactivities of anti-sperm monoclonal antibodies (MABs)

Tissue and other reactivities observed	Number of MABs tested	Number of MABs reacting
Live human sperm surface, before/after washing, and before/after capacitation	66	32
Human testis, seminiferous tubules only	66	9
Human testis, interstitial compartment only/also	66	24
LDH-C4 neutralizing activity	49	11
Cross-reactivities with other human tissues	60	33

Development of an anti-sperm vaccine

Studies on anti-gamete vaccines, which are likely to be effective prior to fertilization, have been supported by the Task Force in the past (Hjort and Griffin, 1985; Hjort et al, 1985; Mori et al, 1985; Shelton and Goldberg, 1985; Wang et al, 1986) and are being supported currently by several national and international funding agencies (Bronson et al, 1985; Czuppon, 1985; Lehmann et al, 1985; Mathur et al, 1985; Mettler et al, 1985). In order to avoid duplication of effort, the Task Force is not funding a major research programme in this area. However, it is conducting a systematic evaluation of anti-sperm MABs in order to characterize these reagents in terms of their abilities to:

-- react specifically with human late spermatids and mature spermatozoa;

-- cross-react with similar or equivalent cellular stages of spermatogenesis in other mammalian species;

-- interfere with sperm motility or inhibit sperm-ovum attachment and fertilization *in vitro* and/or *in vivo*.

The overall objectives of these studies are similar to those described for anti-trophoblast MABs in the preceding section of this report, namely to identify MABs that can be used to isolate and characterize sperm membrane antigens that represent appropriate candidates for development into anti-sperm vaccines.

Again, as the initial step in this new research programme, the Task Force has carried out a systematic evaluation of a large number of anti-sperm MABs that had already been produced by investigators in the field. The data generated in these studies were reviewed and discussed at the workshop (Anderson et al, 1987), jointly organized by WHO and Family Health International (FHI), and held in Toronto, Canada, in June 1986 in conjunction with the third International Congress on Reproductive Immunology and the sixth International Congress of Immunology. A total of 66 such antibodies, alleged to satisfy the primary criterion of sperm specificity, were obtained from 17 investigators and further char-

acterized, in coded form, in terms of the types of tissue with which they reacted, the location of these reactions within the tissue, and the nature of the putative antigenic material.

Tissue location studies were carried out, by immunofluorescence and immunoperoxidase staining procedures, on human testis sections (to distinguish between reactions with components of the seminiferous tubules and the interstitial compartment of this organ), and on a large number of other normal human tissues and fluids. Some of the MABs were assessed for their abilities to neutralize the enzymic action of the sperm-specific lactate dehydrogenase isozyme, LDH-C4. The results obtained in these preliminary studies are summarized in Table 3.

Species cross-reactivity was assessed against a variety of primates (gorilla, orangutan, rhesus monkey, baboon, chimpanzee, marmoset) and other species (elephant, hamster, rat, mouse, horse, mountain sheep, dog). Eighteen MABs cross-reacted with sperm from at least one non-human primate species and 21 cross-reacted with mouse sperm in at least one laboratory. Wide species cross-reactivities were exhibited by four MABs.

A variety of tests were carried out to assess the inhibitory activity of the MABs on sperm function. These included inhibition of sperm motility, sperm agglutination, sperm immobilization, inhibition of cervical mucus penetration, and inhibition of the penetration of hamster ova by human sperm. The results obtained in these functional tests are summarized in Table 4.

Four laboratories have carried out studies in an attempt to derive information on the physicochemical properties of the human sperm antigens identified by selected sperm-specific MABs. Although the results obtained by these laboratories did not correlate well, the more relevant materials identified in this way have molecular weights in the range 15-30 kDa.

These preliminary studies have identified six MABs, out of the original group of 66, that appear to satisfy the criteria established by the Task Force. In addition, these studies have demonstrated a considerable variability in surface expression of most sperm antigens identified by sperm-specific MABs. This variability may be a product of the wide variation of immunizing materials used by different investigators as well as a reflection of the inherent polymorphism of these antigens. In addition, antigenic expression on the sperm surface can be affected by the known and suspected changes that occur as a result of passive coating with seminal plasma

Table 4. Activities of anti-sperm monoclonal antibodies in sperm function tests

Functional test	Number of MABs tested	Number of MABs active
Inhibition of sperm motility	62	22
Sperm agglutination	55	15
Sperm immobilization	55	8
Inhibition of cervical mucus penetration	35	8
Inhibition of hamster ovum penetration	53	25

components, enzymatic modification as well as the stage-specific structural changes associated with maturation, capacitation and the acrosome reaction.

In view of the confusing and often conflicting data being generated in this area, the Task Force has initiated a sperm antigen classification project. This project is modelled on other WHO-sponsored nomenclature programmes in other fields (for example leucocyte and parasite antigens) and will establish an organized data base and nomenclature system specifically for human sperm antigens. Initially, the work to be carried out will form an extension of the studies already conducted by the Task Force and will involve the collection, banking, distribution and characterization of anti-sperm MABs with a final objective of producing a computerized data base in which each sperm-specific antigen will be identified by a project-assigned WHO code number.

FUTURE DIRECTIONS

The Task Force is proposing to carry out the following activities during the 1988/89 biennium.

Subject to a satisfactory outcome of animal teratology studies, approval will be sought to carry out a limited efficacy evaluation of the current anti-hCG vaccine formulation in fertile women volunteers. In addition, the Task Force will continue with the development of an improved version of this vaccine, using a slow-release delivery system designed to elicit long-lasting immunity from a single course of immunization, as well as the development of a second generation of anti-hCG vaccine that will represent a viable product prototype. This latter work will involve the systematic development and comparative evaluation of additional synthetic peptide immunogens, carrier molecules, adjuvants and delivery systems in order to produce a vaccine that is clinically acceptable, appropriate for product development, and suitable for wide-scale application in family planning programmes.

Antifertility studies will be carried out in animals to evaluate prototype anti-trophoblast vaccines. The antigens to be used in these vaccines will have been identified by characterization of trophoblast membrane extracts and by screening gene libraries from human placentae, using selected anti-trophoblast MABs that satisfy the Task Force's criteria of tissue-specificity, lack of species-specificity, and a function-inhibiting or disrupting action.

The Task Force will continue with its recently initiated programme to classify sperm-specific MABs, with a view to characterizing sperm membrane antigens that might represent suitable candidates for development into anti-sperm vaccines. It is envisaged that this work will be conducted in close collaboration with the Task Force on Methods for the Regulation of Male Fertility and with other agencies active in this area. In addition, developments in the field of zona pellucida immunobiology, local (secretory) immunity and 'basic vaccinology' will be monitored in terms of their relevance to the development of antifertility vaccines.

The Task Force is planning to convene a meeting on vaccine safety in order to develop guidelines for the preclinical and clinical safety evaluations of vaccines in general and for antifertility vaccines in particular. It is envisaged that this meeting will be organized in conjunction with other vaccine development programmes within WHO and with other agencies interested in the development of antifertility vaccines. Basic and clinical scientists, representatives of national drug regulatory authorities, the pharmaceutical industry and consumer groups will be invited to participate in this meeting.

The Task Force will continue with its coordination activities in the field of fertility regulating vaccines. As in the past, this coordination will include participation in Task Force Steering Committee meetings by representatives of CONRAD, the Population Council, the Indian National Institute of Immunology and the US National Institute of Child Health and Human Development; the convening of inter-agency consultations; organization of symposia; and the joint funding of research projects of mutual interest.

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Male Fertility Regulation

G. M. H. Waites

Summary

In the quest for appropriate family size, it is surely axiomatic that the male partner should be able to share the benefits and risks of whatever contraceptive strategy the couple may follow. Women, however, may argue that a proper sharing will not occur until men everywhere demonstrate a greater readiness to accept their responsibility in this area. Yet in developed countries, male methods of contraception, i.e. condoms, vasectomy and withdrawal, account for 20-80% of the contraceptive strategy among couples of reproductive age and decision making by men is a prominent feature of family planning in certain cultures (Potts, 1986). Furthermore, nearly 50 million men in the world have opted for vasectomy, more than 10 million in the Sichuan Province of China alone, and it is the method of choice for over 10% of couples practising contraception in North America.

For men to have as wide a choice as women, however, there is clearly a need for a greater variety of safe antifertility methods capable of reversibly suppressing sperm production or sperm function in men without interfering with their libido or any other feature of their health status. Thus, while continuing to improve existing methods, the Task Force on Methods for the Regulation of Male Fertility is also aiming to provide this choice through a research programme with involvement in the following broad areas:

-- improved vas occlusive procedures, and the assessment of the safety, efficacy and reversibility of surgical and chemical methods of vas occlusion;

-- suppression of sperm production by hormonal means, including the development of long-acting androgen supplementation;

-- evaluation of the need to achieve azoospermia or severe oligospermia in male fertility regulation and the development of *in vitro* tests of sperm function;

-- the search for drugs with an action on spermiogenesis or on sperm maturation in the epididymis, including collaborative chemical synthesis and screening programmes with other public sector agencies;

-- collaborative research on new leads of plant origin, specifically *Tripterygium wilfordii*, a putative antifertility agent of Chinese origin;

-- the initiation of a mission orientated basic science research programme to focus modern techniques in cell and molecular biology onto defined targets in spermiogenesis and sperm maturation that are susceptible to interference.

In addition to its own research strategy, the Task Force is active in encouraging the interest, raising the awareness and increasing the competence of scientists to do good research. Examples of how this is effected are as follows:

-- the preparation and distribution of publications for standardization of methods in andrology, e.g. the *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction (1987)* and "*The zona-free hamster oocyte penetration test and the diagno-*

sis of male fertility" (1986);

-- involvement in national and international meetings on andrology and in the review, with other agencies, of research advances in male fertility regulation which have led to publications reaching a wide audience;

-- improvements in research capability by means of andrology workshops and through the training of selected research personnel in both the clinical and basic science aspects of andrology.

Finally, in recognition of the need for a broad approach, from clinical trials to goal orientated basic research, and of the budgetary constraints existing everywhere, the Task Force has developed its research strategy through collaborative activities with Ministries of Health and with several public sector agencies, including: the Contraceptive Development Branch, Center for Population Research of the National Institutes of Child Health and Human Development (CDB/NICHD); International Organisation for Chemical Sciences in Development (IOCD); Contraceptive Research and Development Program (CONRAD); Population Council; Rockefeller Foundation; Family Health International (FHI); and the World Federation of Health Agencies for the Advancement of Voluntary Surgical Contraception.

INTRODUCTION

Men have only a limited choice for the regulation of their own fertility: withdrawal; the use of condoms; and sterilization by vas deferens occlusion. Even with this major limitation, it is estimated that almost one-third of all couples around the world rely on methods requiring male cooperation (United Nations, 1984). Although a major proportion of the Task Force activities in the period 1982-1986 has gone towards establishing the safety and efficacy of immediate methods (for instance, vasectomy), it has also sustained strategies to provide safe and reversible methods based on hormonal, chemical or physical (see Kandeel and Swerdloff, 1988) means. Until these are available, men will continue to be deprived of the full range of options to enable them to share more in responsible family planning.

It would be naive not to acknowledge that the task is difficult. The long-term suppression of sperm production, sufficient to render men infertile with no adverse side effects and with certain reversibility, is a daunting objective indeed. Yet, the Task Force's current strategy is based on a realistic assessment of the opportunities now offered by recent advances in many relevant areas of preclinical and clinical research.

A number of significant developments may be summarized here to lend substance to the optimistic tone of this Introduction. Some derive directly from Task Force-funded research and others have been facilitated, to a greater or lesser extent, by the Task Force's activities. For example, during the 1986/87 biennium:

-- a major collaborative, retrospective, epidemiological study carried out by Chinese scientists in Sichuan has confirmed the long-term safety of vasectomy (Tang et al, 1986, 1988);

-- innovative developments in vas occlusive procedures have emerged in China, including one giving preliminary evidence of reliable reversibility;

- studies have been carried out on non-human primates which suggest that LH-RH antagonists may be more effective than LH-RH agonists in suppressing spermatogenesis and the clinical testing of selected LH-RH antagonists has been initiated;

-- evidence has been obtained that a long-acting testosterone ester can restore and maintain circulating testosterone levels in the normal range for up to four months in non-human primates;

-- remarkable developments have occurred in the identification of inhibin, including the production by gene technology of human inhibin;

-- preliminary evidence has been generated from two sources, to indicate that residual sperm produced by men suppressed to severe oligozoospermia by means of steroid treatment are functionally impaired;

-- evidence has accumulated from the clinical follow-up of male patients and from animal experiments that extracts of the plant, *Tripterygium wilfordii*, have an antifertility effect which may occur primarily at the level of the epididymis;

-- basic scientists have expressed a commitment to the field as witnessed in a Symposium on "Cellular and molecular events in spermiogenesis as targets for fertility regulation" and the response to an advertisement for research proposals in the same area;

-- the approximately threefold increase in the number of scientists directly involved with the Task Force from 1983 to 1987;

-- collaborating agencies have become heavily involved in research on the regulation of male fertility;

-- remarkable progress has been achieved in the global promotion of the participation of men in family planning programmes (see Population Report, 1986);

-- some pharmaceutical companies have demonstrated their willingness to lend their expertise and, in a few cases, to provide material assistance to research on male fertility regulation.

THE PAST: 1950-1985

The earliest attempts to control male fertility by hormonal or chemical means involved negative feedback suppression of pituitary gonadotrophin secretion by increasing the circulating levels of testosterone (Heckel et al, 1951). Thereafter, a variety of nonhormonal agents with an action on the testis were investigated: bis (dichloroacetyl) diamines; various alkylating agents; 2, 4-dinitropyrroles and others which reached the stage of clinical testing before being abandoned because of adverse effects, e.g. nitrofurans and the bis (dichloroacetyl) diamines. During the same period, progestogens developed as oral contraceptives for women, were tested for their ability to inhibit pituitary function in men and a nonsteroidal pituitary inhibitor, methallibure, also reached clinical trials. In 1969, the first drug with an action on the epididymis, 3-chloro-1,2 propanediol (α-chlorohydrin) was described. Cyproterone acetate (CPA) released from silastic capsules was also claimed to interfere with epididymal function in the rat (for references, see Vickery et al, 1986).

Apart from the studies with steroids, largely performed in departments of clinical endocrinology, many of the other compounds were the by-products of research carried out by pharmaceutical companies. The Population Council, National Institutes of Health, (NIH) and the national medical research councils were supporting basic research in general reproductive physiology and relevant studies were being conducted in some animal research institutes. Although funding for research in reproduction was poor and few laboratories were specializing in male reproductive function, the stage was set for a concerted effort for the development of a coherent male antifertility strategy.

The Task Force was established in 1972, initially with the name of "Methods for the Regulation (in the Male) of Fertilizing Ability of Sperm". The mandate for the Task Force did not include male infertility, immunological research or sperm migration and survival in the female tract which were to be covered by the formation of other Task Forces. By 1973, the present name of the Task Force had been adopted and its research objectives covered two broad areas:

-- the development of methods that inhibit spermatogenesis, which included the initiation of the first WHO clinical trial to examine the efficacy of a gestagen-androgen combination, and the initiation of research on the isolation of 'inhibin' from the testis;

-- the development of methods of interfering with sperm maturation, which included two components, namely the development of *in vitro* systems to test the fertilizing ability of mammalian sperm, and the selective interference of sperm maturation through a better understanding of the epididymal environment.

From these early beginnings, the Task Force strategy developed along lines described in the Special Programme Annual Reports and in a review of the Special Programme, Diczfalusy (1986).

A major feature involved the suppression of gonadotrophin secretion together with concomittant androgen replacement and was examined in dose-finding clinical studies using steroidal drugs which were readily available (see WHO Special Programme of Research, Development and Research Training in Human Reproduction, *Fourteenth Annual Report*, 1985). The most effective combination was found to be depot medroxyprogesterone acetate (DMPA, 200 mg per month) and testosterone enantate (200 mg per month). While consistent azoospermia was achieved in only half of the men, most of those rendered oligozoospermic had sperm counts of less than 5 x 10^6 per ml. With cyproterone acetate (CPA), consistent reductions of sperm counts, of the proportion of normal spermatozoa in the ejaculate and of sperm migration through cervical mucus were demonstrated, but there were also signs of androgen deficiency (Roy, et al, 1976). Indian scientists, considering CPA to be more a gestagen than an anti-androgen, initiated studies on the effect of the combination of cyproterone acetate with testosterone enantate with support from the Indian Council of Medical Research (ICMR) (Roy, S., unpublished observations).

Recognizing the need for long-acting androgen replacement, testosterone ester derivatives synthesized in the WHO Steroid Synthesis Programme were screened in a collaborative programme with National Institute of Child Health and Human Development (NICHD) and several were found to be more potent than testosterone enantate. The 'lead' androgen has since been identified as testosterone trans-4-n-butyl cyclohexylcarboxylate (code name: 20 AET-1, see page 205).

A consultation to explore the possible effects of steroidal methods of fertility regulation on prostate function was held in 1978 and concluded that while the causes of benign prostatic hypertrophy and prostatic cancer are unknown, androgens may play a permissive and/or regulatory role once either process has been established. It was suggested that the upper age limit for men in clinical trials involving androgens should be 45, given the greater incidence of prostatic dysfunction in older men.

In 1972/73, at a time when there was still uncertainty about the role of follicle stimulating hormone (FSH) in the regulation of male fertility, the Task Force nevertheless accepted the challenge and hope offered by inhibin for the suppression of a single gonadotrophin which, if successful, would provide an antifertility agent based on a natural hormone and which did not need androgen replacement. A coordinated research programme was initiated in nine laboratories to identify and characterize the testicular source of proteins capable of modulating the secretion of FSH. Major difficulties were encountered concerning the identification of appropriate assay systems, the widely differing molecular characteristics of the various fractions obtained by different laboratories and the lack of sufficient material to establish a working standard. There was as yet no clear demonstration that inhibin could suppress FSH and thereby inhibit spermatogenesis and male fertility *in vivo*. Some appreciation of the achievements of the Task Force supported research can be derived from the Proceedings of a Symposium on inhibin, FSH and spermatogenesis held in Cambridge, UK, July 1978 (Setchell and Weir, 1979).

Task Force supported studies based on immunization procedures suggested that FSH may not be essential for the maintenance of spermatogenesis in adult rats or in rhesus monkeys. Although active immunization against FSH did have an early inhibitory effect, it eventually disappeared with time (Srinath et al, 1983; Nieschlag, 1986). On the other hand, passive

and active immunization of bonnet monkeys resulted in azoospermia or oligozoospermia and in some cases in infertility (Moudgal, 1981; Moudgal et al, 1987). These latter studies are ongoing as a part of the Indo-US Science and Technology Initiative.

In the early 1970s, FSH was emerging as the major metabolic stimulant to the testis. Procedures of cell separation, primary cell culture and the collection of tubular fluid, established that the Sertoli cell was: the target cell for FSH; the intermediary in metabolic support of the germinal epithelium; the originator of tubular fluid and of a multitude of secretory products; and a key feature of the 'blood-testis barrier' (for reviews, see Fritz, 1978; Setchell, 1980; Waites and Gladwell, 1982).

During 1974/78, the Task Force, attracted by the concept that some of the secretory proteins of the testis could be targets for male fertility regulating agents, supported studies on an androgen binding protein (ABP) secreted by the Sertoli cell and transported to the caput epididymis. Since all of the actions of FSH on spermatogenesis appeared to be mediated by the Sertoli cell, it was thought that interference with ABP might not only interfere with spermatogenesis but also with the capacity of the epididymis to support sperm maturation. The Task Force studies characterized rat and rabbit ABP, established that antiserum to rat FSH inhibited ABP production and spermatogenesis, and provided new evidence concerning androgen receptors (Hansson et al, 1976). This line of research was terminated in 1978 when it was found difficult to show that the human testis produced ABP and to establish if it differed immunologically and functionally from serum testosterone-binding globulin.

Task Force supported studies on the epididymis yielded new information on the composition of fluid in the epididymis and on the significance of the high concentrations of carnitine and androgen-dependent proteins for sperm maturation. The examination of fluid from different regions of the human epididymal tubule (Bedford et al, 1973) and the assessment of pregnancy outcome following operations for epididymo-vasostomy (Schoysman and Bedford, 1986) established that passage through the proximal region was necessary for the maturation of sperm motility and acquisition of some degree of fertilizing ability.

Studies on α-chlorohydrin and its derivatives were terminated in 1976 because of toxicity. Together with subsequent studies on halogenated sugars, which were also abandoned because of toxicity, these compounds had firmly established in several species, including non-human primates, that drugs existed which could selectively interfere with fertility through inhibiting sperm maturation. The action was due to an effect on the glycolytic ability of epididymally-stored sperm and was reversible. The target was found to be a specific enzyme in the glycolytic pathway of epididymal sperm, glyceraldehyde-3-phosphate dehydrogenase (for review, see Ford, 1982).

When the down-regulatory effects of LH-RH agonists on gonadotrophin secretion were first demonstrated in 1979, the Task Force drafted a clinical trial protocol to explore their value as male antifertility agents. The Toxicology Group of the Programme felt unable to approve a duration of treatment longer than two weeks with these largely untested compounds and as this was insufficient to establish the antifertility potential of these agents, the study was not initiated. Since then, the efficacy of LH-RH agonists in suppression of sperm production has been tested in clinical trials conducted by other agencies (for reviews see Swerdloff et al, 1985; Knuth and Nieschlag, 1987; Bhasin et al, 1987).

The Task Force's activities were temporarily suspended in the period 1980/82. During this period, however, the Programme did not abdicate its involvement in the field of andrology. The first edition of the *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction* was published in 1980, the screening of long-acting androgen esters was continued and the first two Andrology Workshops were conducted in Singapore. Collaboration was initiated with the People's Republic of China in the development of capabilities for basic, clinical and epidemiological research in male fertility control and this resulted

in immediate major consequences related to gossypol and vasectomy. But equally important, these developments heralded the start of a process of institution strengthening in China which was to go some way towards allaying two of the concerns expressed earlier about male research, i.e. the relatively small number of personnel and well-founded centres working in the field.

The development of its research strategy in the period 1982/85 and the background from which the research progress in the biennium 1986/87 grew are given in the report of the Task Force in the Special Programme's *Fourteenth Annual Report* (WHO Special Programme of Research, Development and Research Training in Human Reproduction, 1985, pp. 72-86; and by Waites 1986). The Proceedings of an International Workshop on male contraception, sponsored by the Program for Applied Research on Fertility Regulation (PARFR) provide comprehensive reviews of the whole field (Zatuchni et al, 1986).

THE PRESENT

There have been a number of significant developments in the last two years which have helped to shape the current research strategy of the Task Force.

Vas occlusive procedures

Vasectomy

Vasectomy and cardiovascular status (collaborative study with the Sichuan Family Planning Institute)

Major concerns about the possible health consequences of the immune response to vasectomy were raised by studies on animal models (Alexander and Clarkson 1978; Clarkson and Alexander, 1980). These stimulated a series of epidemiological studies of which this one was initiated in 1983. The analysis of the considerable amount of data collected on the health status of 4,596 vasectomized and 4,340 non-vasectomized men from eight rural communes in Sichuan, was reported at the XII World Congress on Fertility and Sterility (Tang et al, 1986) and are to be published (Tang et al, 1988). In summary, the mean duration since vasectomy was 14.5 years with range being 10 to 25 years. At the time of evaluation, the vasectomized men were generally healthier than the non-vasectomized for a wide range of health indicators, including clinical signs of cardiovascular disease, resting ECG changes, positive ECG changes following a maximal stress test, or fundus abnormalities. The lack of association between vasectomy and cardiovascular disease noted in India, Europe and the USA (see Massey et al, 1984; Petitti, 1986) is supported by this study conducted in a population with a naturally low prevalence of cardiovascular disease and risk factors.

Supplementary publications analysing various elements of the main study, e.g. retinopathy findings, lipid analyses are being prepared by the Chinese investigators.

Vasectomy and endocrine status

A cross sectional study to investigate the long-term effects of vasectomy on reproductive hormone status showed no differences in testosterone, LH, FSH and prolactin levels between 505 men vasectomized 1-25 years previously and 298 age-matched non-vasectomized controls (Peng et al, 1987).

Vasectomy and prostate function

A study involving three-dimensional ultrasound tomography reported that the peri-urethral portion of the prostate of men one year after vasectomy was significantly reduced in size compared to before the operation (Jakobsen and Juul, 1985). However, a Task Force-supported retrospective follow-up study by the same investigators, on 56 men vasectomized approximately eight years previously compared to 56 age-matched controls, failed to find any changes in prostatic volume or pattern of micturition although the semen volume was slightly but significantly reduced after vasectomy (Jakobsen et al, 1987 and 1988).

Immunology of vasectomy reversal

There has been considerable speculation concerning the role of autoantibodies to sperm in preventing subsequent restoration of fertility after vasovasostomy (for review see Silber, 1986). A study in the Collaborating Centre in Seoul on the predictive significance of the immune status of vasectomized men for success of subsequent vasovasostomy has shown that, of 34 vasovasostomy cases, 31 (91%) had sperm in the ejaculate and 10 (30%) achieved pregnancy. Patients whose partners became pregnant had low antibody titres suggesting that high antibody titres may be associated with lack of return to fertility. The significance of immune status for pregnancy outcome should be clearer when more cases are available (Lee et al, 1988). The protocol used in this WHO study will form the basis for similar studies to be conducted in centres in China.

Percutaneous vas occlusion

The development of non-surgical techniques for vas occlusion which avoid a skin incision are likely to be more acceptable in some cultures and may be easier to reverse. A description of Chinese techniques of non-incision vasectomy involving special clamps and other procedures and of the percutaneous injection of a sclerotising solution (n-butyl-2-cyanoacrylate and phenol) into the vas deferens appeared in the publication of a joint WHO-PRC symposium (Li and Zhu, 1985). In addition, Xiao (1987) recently reviewed chemical methods for vas occlusion in use in China and described the use of 'medical-grade' polyurethane elastomers (MPU) to form plugs. This latter method appeared to offer the possibility of easy reversal. A WHO-site visit team reviewed such methods in Chongqing, Shanghai and Taiyuan in 1987 and made the following recommendations:

-- A protocol should be drawn up to study the immunological and other aspects of vasovasostomy following surgical vasectomy.

-- This protocol should be used in follow-up studies after the removal of the MPU plugs from men in Taiyuan.

-- The availability of other toxicologically-approved materials should be explored in order to study the efficacy and the effect of removal of plugs formed from such materials.

Hormonal suppression of spermatogenesis

The Task Force is continuing actively to pursue endocrine methods of regulating male fertility based on the suppression of gonadotrophins by either gestagens or LH-RH analogues (Bremner and Matsumoto, 1986; Knuth and Nieschlag, 1987; Nieschlag et al, 1987). All such methods crucially depend upon having a slow release, long-acting androgen preparation. The requirements are: to restore systemic levels of androgen to the physiological range following suppression of gonadotrophin secretion; to do so in a non-pulsatile fashion so that spermatogenesis is not restimulated; and to extend the interval between administrations in order to improve acceptability.

Long-acting androgen supplementation

Dose-finding pharmacokinetic studies in two Centres using castrated male rhesus (New Delhi) and cynomolgus (Munster) monkeys showed that with a single intra-muscular injection of testosterone trans-4-n-butyl cyclohexylcarboxylate (20 AET-1), serum testosterone levels could be maintained in the physiological range for up to four months (Weinbauer et al, 1986; Rajalakshmi, M., unpublished observations) whereas currently available long-acting formulations (e.g. testosterone enantate or testosterone cypionate) have a much more limited duration of effect. Subsequent studies indicated that increasing the injected dose of 20 AET-1 extends the duration of action. In contrast to testosterone enantate, 20 AET-1 does not produce supra-physiological peaks of serum testosterone and appears to be suitable for human application.

In preparation for clinical trials of this compound, 300 g of 20 AET-1 has been produced under Good Manufacturing Practice (GMP) conditions and will be ampouled as an aqueous milled suspension. The WHO Toxicology

Group requested information on the metabolic fate of the ester side chain and the 'pro-drug' nature of 20 AET-1. This information is being collected. Meanwhile, the protocol for the Phase I human study has been drafted.

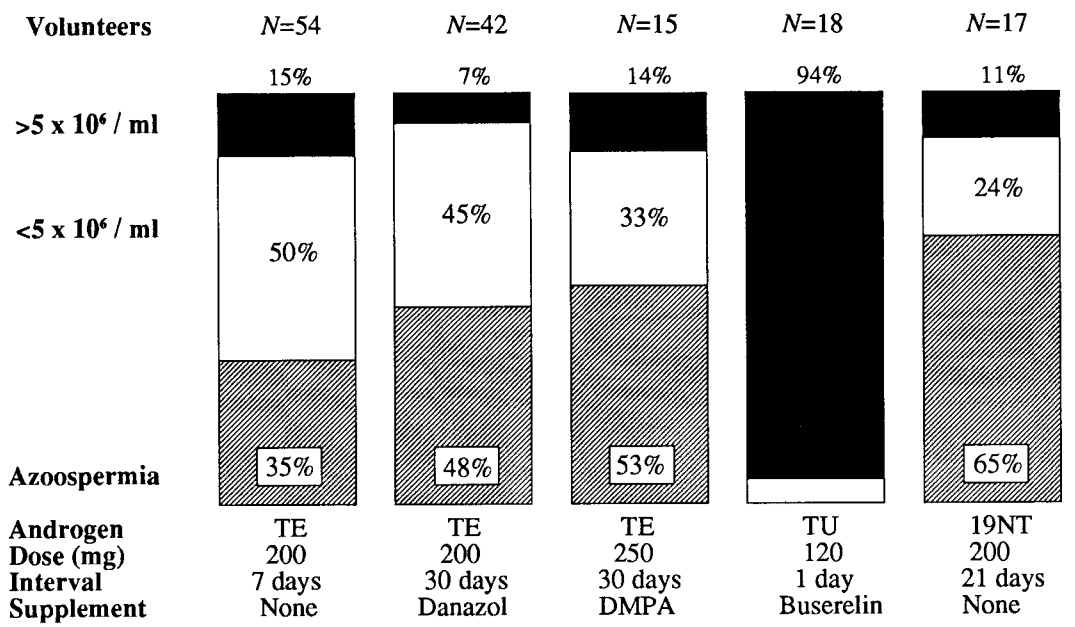
Androgen-gestagen combinations

As stated earlier, the most effective steroid combination, DMPA + testosterone enantate (TE) yielded only approximately 50% azoospermia in clinical trials. The remaining men had severe oligozoospermia. Recently, it was shown that a long-acting ester of 19 nortestosterone (19NT) alone (Schurmeyer et al, 1984; Knuth et al, 1985) or in combination with DMPA (Knuth, U.A. and Nieschlag, E., unpublished observations) was more effective in producing azoospermia than when TE was used as the androgen component (Fig. 1). However, the number of subjects in these studies did not allow statistically valid conclusions to be made. A multicentre trial is planned in three countries to establish if androgens of different pharmacokinetic characteristics influence the sperm-suppressing effect of DMPA.

LH-RH analogues in combination with improved androgen supplementation

While the current view favours the use of LH-RH antagonists for male fertility control, there are two issues that need to be addressed in con-

Fig.1. Comparison of success rate in representative experimental trials for male fertility regulation using different ways to suppress gonadotrophins. 'Androgen' indicates substance used to maintain virility: TE = testosterone enanthate, TU = testosterone undecanoate, 19NT = 19 nortestosterone hexyl oxyphenyl-proprionate. 'Interval' describes the frequency of the androgen administration per multiple days. 'Supplement' lists the antigonadotropic substance given in addition (From Knuth and Nieschlag, 1987, which should be consulted for the references)



sidering both agonists and antagonists for this purpose: reversibility of testicular suppression after long-term use; and androgen supplementation.

Obtaining information on the reversibility of testicular suppression after long-term use of these compounds is difficult in that the rat appears to be an unsuitable model; available data from prostatic carcinoma patients under LH-RH agonist treatment are conflicting; and testicular biopsies cannot be performed on healthy men for ethical reasons. A small-scale study in rhesus monkeys showed complete reversibility after 20 months suppression (Weinbauer et al, 1987). A Task Force-commissioned study on the reversibility of testicular function after three-year suppression with an LH-RH agonist (Buserelin, Hoechst) is being conducted in bonnet monkeys in Bangalore, India.

Concerning androgen supplementation, there is a possibility that the forms of testosterone replacement used may be attenuating the suppressing action of LH-RH agonists on sperm production. For this reason, the Task Force is exploring whether or not other androgens exhibit this attenuating effect. Bearing in mind the lack of aromatization in its metabolism, the Steering Committee is exploring if dihydrotestosterone (DHT) is a better candidate. The Task Force has therefore initiated a study in cynomolgus monkeys to compare the effects of DHT-enantate (DHT-E) or testosterone enantate (TE) in combination with Buserelin on spermatogenic suppression and has drafted a protocol for a Phase 1 study comparing DHT-E and TE in normal and hypogonadal men.

LH-RH antagonists are believed to be better candidates for suppressing sperm production than LH-RH agonists (see Knuth and Nieschlag, 1987; Nieschlag et al, 1986). Since the LH-RH antagonists so far developed were not free from side effects related to histamine-release, the Steering Committee felt that it was too early to carry out clinical studies with them. However, it is maintaining a close surveillance of the field and the Programme is negotiating for the collaborative development of safe LH-RH antagonists for use in clinical trials.

Role of FSH in male fertility

Immunization against FSH

There is evidence that suppression of FSH production alone would not be likely to suppress spermatogenesis completely in humans (Bremner and Matsumoto, 1986). However, in recent studies supported by the ICMR involving active immunization against FSH in bonnet monkeys, the monkeys were often not azoospermic and sometimes produced relatively large numbers of spermatozoa but the semen quality was poor and they were infertile in mating tests (Moudgal et al, 1987). Thus, the influence of FSH on the functional maturation of sperm is an important consideration which might provide a lead for contraceptive development.

Provision of an International Research Standard for Inhibin

The possibility of specifically suppressing FSH by means of a single natural hormone, inhibin, remains enticing despite all the difficulties in its isolation and characterization (see Findlay 1986; de Kretser et al, 1987). However, the full amino acid sequence of two forms of inhibin of follicular fluid origin, each with a relative molecular mass of 32kDa, has been reported (Ling et al, 1985; Mason et al, 1985; Miyamoto et al, 1985; Forage et al, 1986). Each form is composed of two cross-linked subunits, a larger common α-subunit (M_r, 18kDa) and a smaller ß subunit (M_r, 14kDa) which exists in two forms $ß_A$ and $ß_B$ depending on the identity of the terminal amino acid. Recent unexpected findings (Ling et al, 1986; Vale et al, 1986) suggest that 28kDa polypeptides composed of the ß dimers are potent and specific *stimulators* of FSH secretion in *in vitro* systems. Thus the ß dimers of inhibin appear to constitute a third, stimulatory, factor to add to the inhibitory factors of steroids and inhibin itself in the gonadal feedback regulation of FSH secretion. There is considerable homology between inhibins of human, bovine and porcine origin and there is a degree of homology with two other regulatory peptides: Transforming Growth Factor ß (TGFß) and Mullerian Inhibitory Substance (MIS). All are substances of

Fig. 2. Advertisement for the WHO-NIH International Research Standard for Inhibin

RESEARCH STANDARD FOR INHIBIN (PORCINE)

As a result of a WHO/NIH Collaborative Programme, a partially purified extract of porcine follicular fluid has been ampouled (code 86/690) as a Research Standard for bioassays and immunoassays of Inhibin. This is available on written request (outlining the intended use of the standard) sent to **Dr. J.M. Zanelli, Division of Endocrinology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK.** A nominal handling charge will be payable.

potential therapeutic interest (reviewed by McLachlan et al, 1987).

Although these developments offer the possibility of producing the components of human inhibin by genetic engineering techniques in sufficent quantities to test their antifertility potential (see Forage et al, 1987; Mason et al, 1987), the Task Force has decided not to re-enter the field which it did so much to stimulate until such preparations are available. However, in order to have a coordinating and standardizing role, the provision of an international research standard and reagents for the establishment of a radioimmunoassay for inhibin were considered to be an appropriate contribution to the field.

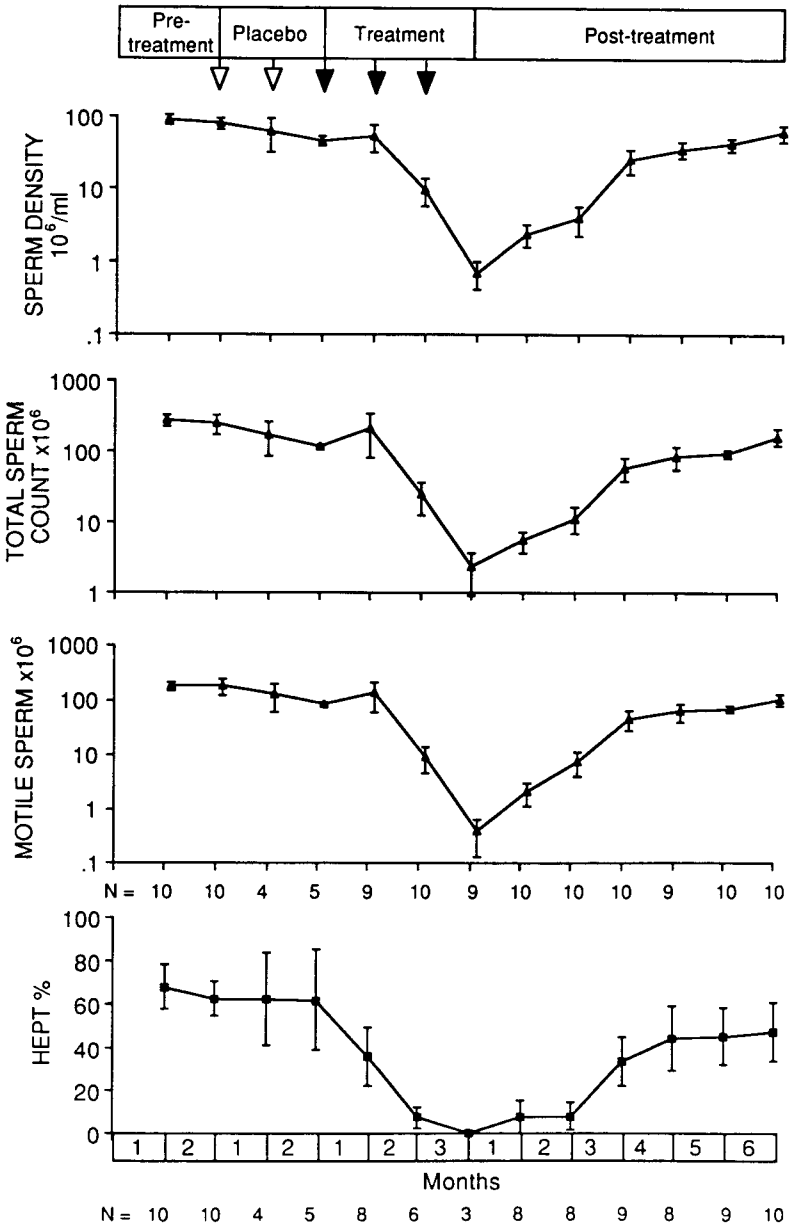
In a collaborative effort with the NICHD, such a standard was established during 1986/87. The source of the starting material was porcine follicular fluid (pFF) and the extraction procedures were those described by Gordon et al, 1986. The potency was assessed in the *in vitro* pituitary cell assay in two laboratories. The material was checked for stability, the quantity per ampoule was decided and a unitage assigned. Finally, ampouling was carried out by the National Institute for Biological Standards and Control, London (a WHO collaborating centre) and the standard was advertised for distribution (Fig. 2). An international collaborative study, which will also include other ampouled preparations of human, bovine and porcine inhibins, is being organized to assess the suitability of the ampouled porcine inhibin (code name 86/690) as an international research standard (see Waites et al, 1987).

Functional capacity of residual sperm

The question of whether or not men suppressed to severe oligozoospermia remained fertile has been relevant ever since it was appreciated that universal azoospermia was going to be hard to achieve by any hormonal means. Recently, there is evidence from two Task Force-supported studies that residual sperm from men suppressed to severe oligozoospermia by DMPA + TE treatment (Wu, F. W. C. and Aitken, R. J. unpublished observations) or TE alone (Bremner W.J. and Matsumoto A.M., unpublished observations) have impaired function when tested in the zona-free hamster oocyte penetration assay (Fig. 3). If sperm from such subjects are unable to fertilize the ovum, this would open up the entire field for the development of hormonal agents which do not produce complete azoospermia. However, since the predictive value of *in vitro* laboratory tests of sperm function is not universally accepted, the field trial approach was the only option to answer this question.

Therefore, the Task Force has initiated a multicentre field trial involving 240 couples. The first part of the study involves the establishment of azoospermia following dosing with TE, followed by a field trial with those men having unprotected intercourse with their partners. The criterion for exclusion of subjects if sperm appear in the ejaculate to concentrations greater than 1×10^6/ml was agreed. If this trial is suc-

Fig. 3. Changes in sperm production and in characteristics related to sperm function in the semen of normal men during (*a*) control (pre-treatment and placebo); (*b*) treatment with 3 intra-muscular injections of 200 mg depot-medroxyprogesterone acetate and 250 mg testosterone enantate (↓); and (*c*) post-treatment periods (HEPT% refers to % penetration in the zona-free hamster oocyte assay) described by Aitken et al, 1983 (from Wu, F. W. C. and Aitken, R. J. unpublished observations)



cessful in terms of an acceptably low pregnancy rate, a crucial subsequent trial will be carried out involving oligozoospermic men with higher sperm counts (<5 million/ml). The Task Force believes that the questions to be answered by this study are pertinent to the whole concept of endocrine approach to fertility control. It should be noted that T.E. was chosen only as the means to suppress sperm count and not as a male antifertility agent.

Improving methods for the evaluation of sperm function

The second edition of the *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction* was published in 1987 taking into account recent technical improvements and in the interests of standardization of methods in this area. In addition, the report of the consultation on the hamster oocyte assay was issued in 1986 which contained a protocol to enable standardization of this test (WHO, 1986). Finally, advanced workshops on sperm function are being planned for the training of scientists in the developing countries.

Collaborative research on *Tripterygium wilfordii*

The extract of the root of *Tripterygium wilfordii*, in widespread use in China as a herbal medicine for the treatment of dermatological conditions and rheumatoid arthritis, was shown to have a reversible antifertility action in rats in a Task Force-supported study (Qian et al, 1986). The historical, medical and chemical background to this observation has been reviewed (Qian, 1987a, b).

The Programme entered into a collaborative research programme on *Tripterygium wilfordii* with the Jiangsu Family Planning Institute. The PRC Ministry of Health and the Jiangsu Provincial Government agreed to provide 500g of the root extract of *Tripterygium wilfordii* free of charge to assist the chemical isolation work which was initiated in a centre in London with the assistance of a WHO research trainee from the Jiangsu Family Planning Institute.

The chemical isolation and purification of several fractions from tablets was achieved; freeze-dried residues of these fractions have been tested by the Nanjing collaborating institute and some have been found to have an antifertility action in rats. A Chinese chemistry laboratory will collaborate in the isolation of active principles to be screened in Nanjing. Patients who had received *Tripterygium wilfordii* tablets as treatment for mild dermatological conditions, will be followed up immediately after cessation of the treatment in order to evaluate the recovery of spermatogenesis.

Post-meiotic and post-testicular maturation

Studies carried out with α-chlorohydrin and 6-chloro-6-deoxysugars in animals and with sulfasalazine in humans (see Giwercman and Skakkebaek, 1986) established that drugs could be found which would have a post-testicular action (Ford and Waites, 1986; Waites, 1987). The Task Force had long recognized that such an action would have several advantages. Interference with the function of sperm after they have left the testis would not disturb spermatogenesis, libido or any other hormonally-related event: the effect would be rapid in onset, and, on withdrawal of the drug, normal sperm would return quickly to the ejaculate (Cooper et al, 1986).

The Task Force has supported three main lines of research in this area:

-- the continuation of studies on the role of the epididymis in sperm maturation, including the definition of appropriate screening procedures for drugs with an epididymal action;

-- the development of collaboration with the International Organisation for Chemical Sciences in Development (IOCD) in the synthesis and screening of analogues of known drugs with a post-testicular antifertility action, including halo-hydrins and halo-sugars;

-- the development of an 'open' programme of basic science research drawing on expertise from outside the field of reproductive biology

Fig. 4. An advertisement which appeared in scientific journals to invite proposals for research on spermiogenesis and sperm maturation

WORLD HEALTH ORGANIZATION

SPECIAL PROGRAMME OF RESEARCH, DEVELOPMENT AND RESEARCH TRAINING IN HUMAN REPRODUCTION

RESEARCH ON SPERMIOGENISIS AND SPERM MATURATION

The WHO Task Force on Methods for the Regulation of Male Fertility is concerned with the promotion of research and development of existing and new methods of male fertility regulation. The post-meiotic development and epididymal maturation of sperm represent enticing options for drug regulation.

The Task Force wishes to promote basic research in this general area, including studies on spermiogenesis because there are unique processes in the post-meiotic stage of evolution of the haploid spermatid e.g. the development of the acrosome and of the sperm flagellum.

Interested scientists are invited to submit research proposals identifying topics for investigations; applications from developing country scientists would be especially welcome. The funding available is limited, so that most grants are not likely to exceed US$ 10,000 for one year in the first instance. The collaborative involvement of scientists outside the field of reproductive biology willing to take part in inter-disciplinary transfer of their techniques would be a welcome feature and 'start-up' grants to achieve this would be considered.

Please send a well-focused summary (not more than 1 page) including a provisional budget to the address below, and if this is considered appropriate, guidelines for the formal application will be sent. The proposals will be dealt with in confidence and a peer-review procedure will be followed.

The Task Force Manager
Methods for the Regulation of Male Fertility,
Special Programme of Research, Development
and Research Training in Human Reproduction,
World Health Organization,
1211 Geneva 27, Switzerland

and from the area of molecular biology.

Interference with the post-testicular maturation of spermatozoa

A study in Hong Kong is applying a unique range of techniques in an attempt to interfere with the fluid environment of the epididymis by inducing secretion of chloride from the blood to the lumen; and to induce the transport of drugs into the epididymal lumen in order to affect the spermatozoa. The use of culture and perfusion techniques for the epididymis and isolated epithelial cells has yielded basic information on the nature of fluid and electrolyte secretion in this tissue (Huang and Wong, 1988; Wen and Wong, 1988; Wong, 1988a,b; Wong and Chan, 1987; Wong and Fu, 1988; Wong et al, 1987b). In parallel studies, a number of sulfonamide analogues have been shown to have an antifertility action which may be mediated through spermatozoa stored in the epididymis. Some of these drugs are specifically accumulated within the lumen of the epididymis (Qiu and Wong, 1985; Wong et al, 1987a).

Synthesis and screening of drugs with a post-testicular action

A joint consultation between the Task Force and the IOCD in July, 1984, established a strategy whereby the latter would develop a synthesis programme for the production of analogues of α-chlorohydrin and 6-chloro-6-deoxysugars which would be tested in Task Force-NICHD fertility assays. More recently, IOCD has established a synthesis and screening programme for analogues of sulfasalazine and related drugs. Collaborative screening tests have also been undertaken by the Task Force-supported centre in Hong Kong and some of the analogues were found to be active in reducing fecundity.

An 'open' research programme for interfering with spermiogenesis and sperm maturation

In acknowledging that drugs with an action on sperm maturation were largely made available by serendipity, the Task Force became convinced of the need for a coherent research strategy based on mission orientated fundamental biomedical science. In reviewing some tentative targets in the wider span of post-meiotic events in sperm maturation, the Task Force proposed two approaches: the support of a Basic Science Symposium; and the initiation of an 'open' programme of research aimed at interfering with spermiogenesis and sperm maturation.

Basic Science Symposium "Golgi, lysosome and centriole events in early spermiogenesis; targets for male fertility regulation", Mexico, 11-13 March 1987

The objectives of the Symposium, jointly sponsored with Family Health International, were to explore what is known of structures which give rise to the unique features of the spermatid during early spermiogenesis, i.e. the acrosome, centriole and flagellum, with special emphasis on the opportunities which might exist for specific interference. Speakers from outside the field of reproductive biology were invited to participate and techniques from molecular biology, cell biochemistry and pharmacology were discussed in terms of the opportunities they might offer for application to fertility regulation. The participants established a set of recommendations as a basis for a research strategy. The proceedings will be published by the Programme as the first of a 'Basic Science in Fertility Regulation' series (Hamilton and Waites, 1988).

'Open' Programme of Research

In response to an advertisement placed by the Task Force in scientific journals (Fig. 4), 42 summary applications were received of which 14 were selected by a sub-committee on the basis of their originality and promise and a full application invited from the investigators concerned. The Task Force has been impressed by the positive response and the quality of the proposals. Three main areas of research are emerging:

-- spermiogenesis;

Fig. 5 (a). Cumulative recovery rate to threshold spermatogenic function after stopping taking gossypol as an antifertility drug. Figures in parenthesis indicate the follow-up time of the 18 of 46 men who had not recovered (from Meng et al, 1988)

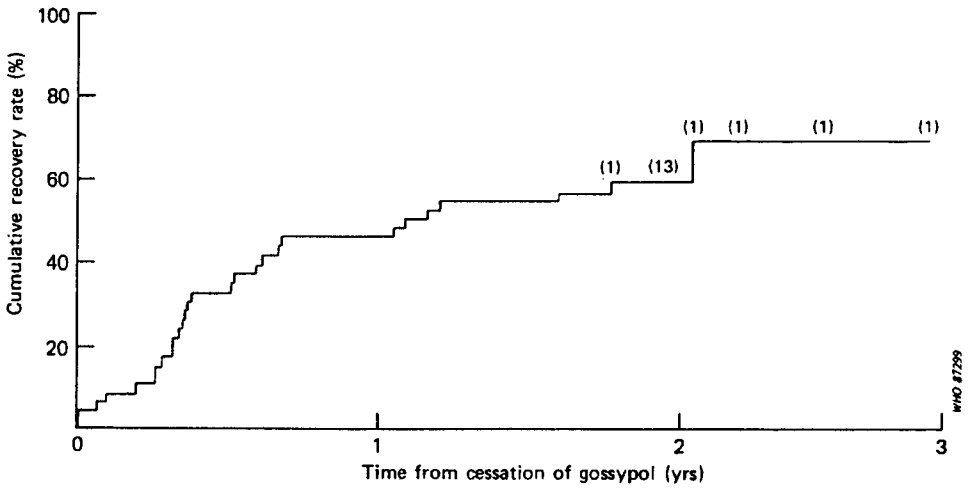


Fig. 5 (b). Cumulative recovery rate according to the duration of gossypol treatment (from Meng et al, 1988)

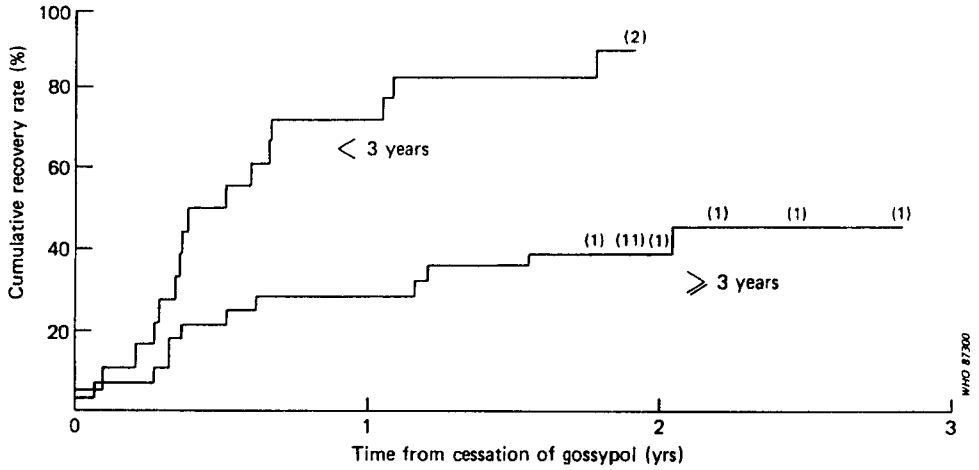


Fig. 5 (c) Cumulative recovery rate according to the total testicular volume at the end of gossypol treatment (from Meng et al, 1988)

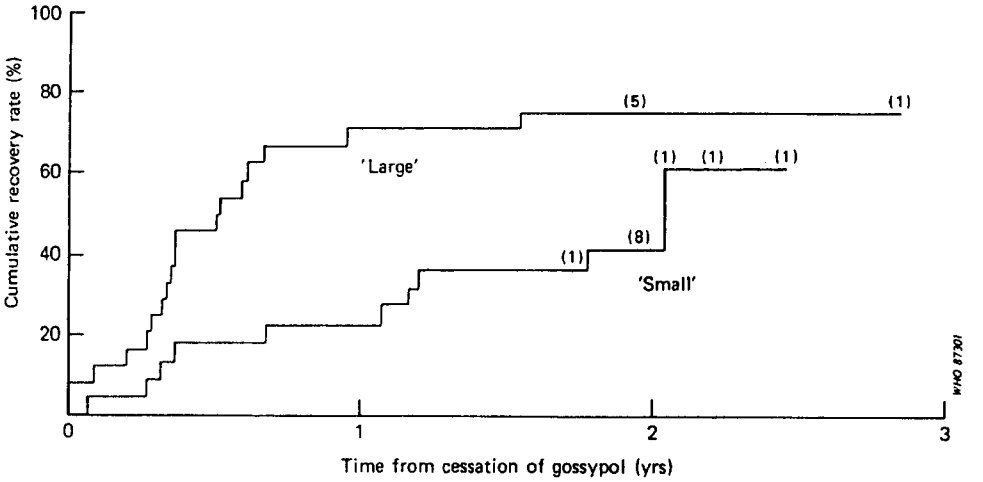
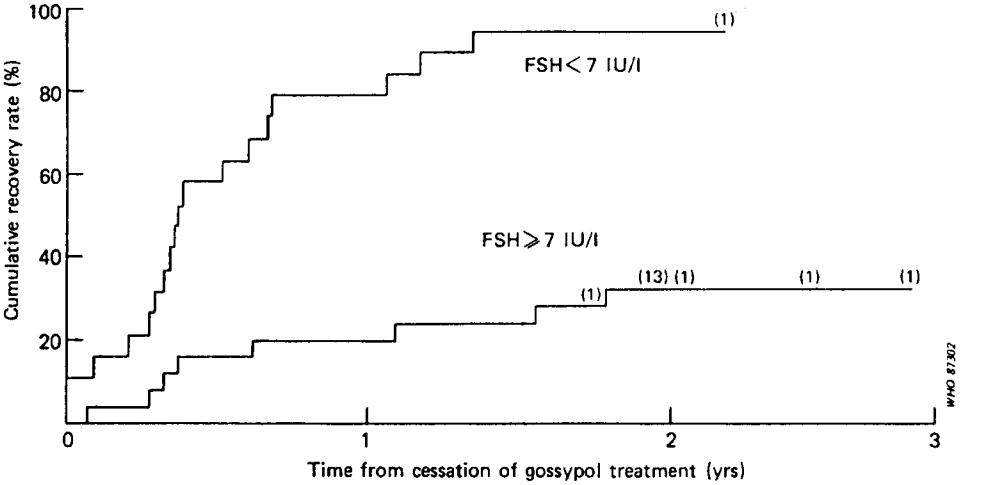


Fig. 5 (d). Cumulative recovery rate according to serum FSH values at the end of gossypol treatment (from Meng et al 1988)



-- the molecular biology of the developing sperm membrane and its role in recognition events in sperm-egg function;

-- sperm surface ion channels and the development of sperm motility.

Gossypol

Animal toxicology

The Task Force completed toxicological studies on (-) gossypol, the gossypol enantiomer with the antifertility action (Matlin et al, 1985; Wang et al, 1987) by attempting to establish the threshold dose which was non-toxic in cynomolgus monkeys. These studies were made possible by the large scale separation of the enantiomers (Huang et al, 1987; Matlin et al, 1987). It was assumed that all the activity of racemic (±)-gossypol in the human resided in the (-)-gossypol portion so that the effective dose of (-)-gossypol was estimated at 0.15 mg/kg/day. Pairs of cynomolgus monkeys were dosed with either 5 mg/kg/day or 4 mg/kg/day; and when each of these dose levels produced toxic effects, 1.5 mg/kg/day (i.e. approximately 10 x human effective dose) was tried. Even at this dose, there were signs of toxicity, in particular gastrointestinal effects (see Heywood, 1988).

The WHO Toxicology Group, which had assessed all the Task Force's safety data on (±)-gossypol acetic acid earlier (see Heywood et al, 1986; Wang and Lei, 1987), reviewed the (-)-gossypol toxicity that had been obtained in monkeys and recommended the discontinuation of further toxicological studies involving gossypol.

Clinical follow-up studies on gossypol

Since 1981, the Special Programme's policy has been not to support clinical trials with gossypol until approved animal toxicology or analogues not having the reported side effects were available. However, the Task Force was concerned to define the principal factors involved in the two major side effects of gossypol in the Chinese clinical trials, namely, lack of reversibility; and hypokalaemia with associated episodes of neuromuscular disorders. For these reasons, institutes collaborating with the Special Programme have undertaken studies on the recovery of men after the cessation of gossypol treatment.

Reversibility

The Family Planning Institutes in Beijing and Nanjing established the baseline control values of semen and reproductive hormones using Task Force protocols (see Zhang, et al, 1985a: Hu et al, 1985). Investigators at these institutions were, therefore, well prepared to follow the recovery of testicular function in men immediately and in the long-term following the cessation of gossypol treatment, again using common protocols. The results were published separately by the investigators (Zhang, et al, 1985b; Zhu, et al, 1985; Hu et al, 1985) and the combined data were analysed in Geneva and a number of variables identified for their predictive association with the degree and time of recovery of spermatogenesis. After 1.9 years follow-up, spermatogenesis did not return to normal in 18 of the 46 men involved (39%) and 10 of the men (22%) remained azoospermic. The failure of recovery from gossypol treatment was strongly associated with longer time of treatment, greater total dose of gossypol, smaller testicular volume, elevated serum FSH concentrations, and, to a lesser extent, with greater body weight (Fig. 5; Meng, et al, 1988).

During an International Symposium on Gossypol Research in Fertility Regulation, held in Wuhan, China, October 1986, which was sponsored jointly with the Ministry of Health, People's Republic of China and the Rockefeller Foundation, several studies were reported by Chinese investigators which dealt with other aspects of the cytotoxic damage to the testis. These included studies on the detailed cytology of the germinal epithelium obtained by biopsy and continuing morphological and functional damage to spermatozoa long after apparent recovery of spermatogenesis. The Proceedings of this Symposium, including the statement on future gossypol research agreed by all participants are to be published in *Contraception* in 1988.

Hypokalaemia

It is now widely agreed that the hypokalaemia occurs as a result of a damaging effect of gossypol on the renal tubules leading to increased loss of urinary potassium (without an increase in plasma bicarbonate levels) and a hypokalaemic effect sometimes lasting beyond the period of gossypol treatment (Qian 1985; Liu et al, 1987; Liu and Lyle, 1987).

Mechanism of action studies

The Task Force has supported studies to define the nature of the metabolic action of gossypol and its enantiomers on testicular cells (Den Boer and Grootegoed 1987, 1988a, b; Tanphaichitr et al, 1988) including two studies by Chinese investigators, some of whom received training through the WHO research training scheme, on the kinetics of transfer of gossypol into the testis and epididymis (Wang, et al 1988; Wang, J.M., Wen, G.Y., Zhang, Z.R., Wu, X.L., Jiang, D.H., Tao, L., Cao, R.Q., and Zhou, Q., unpublished observations). Such studies were chosen for support because of their potential to yield better methods for the *in vivo* screening of gossypol and its derivatives.

Conclusions

In early 1986, the Task Force decided not to initiate new studies on gossypol and to await the conclusions of the Wuhan Symposium. The investigators in the Gossypol Synthesis Programme were informed that none of the 39 analogues tested showed any activity in biological screening tests. Formulation studies were to be continued to attempt to improve the bioavailability of gossypol. In view of the statement agreed at the Wuhan Symposium, of the Task Force's 'follow-up' studies (Meng et al 1988), and of the recommendation of the Programme's Toxicology Group, the Task Force reaffirmed the 1981 decision not to support clinical trials with either racemic or (-)-gossypol. However, minimal activities based on the provision of the enantiomers in ampoules and for mechanism of action studies were to continue. In addition, the gossypol analogues are to be tested for their virucidal and spermicidal effects in screening tests being established by the NICHD.

Andrology Workshops

Two workshops were conducted: Santiago, Chile, 1986 in which 31 participants from 16 centres in Latin America took part; and Tbilisi, U.S.S.R. 1987 in which 33 participants from five centres took part.

THE FUTURE

The Task Force looks with measured confidence to a future of promise growing from the present work and firmly based on the past.

The important issue of the need for azoospermia in a male contraceptive strategy should be answered by the ongoing multicentre trial. This study entered the suppression phase in six of the 10 centres in 1987 and data should be available by 1990 to indicate if the second stage, to define the level of safe oligozoospermia, can be initiated.

The LH-RH antagonists may emerge as a means of safely suppressing gonadotrophin secretion over long periods. If the toxicology being done on two of the antagonists is favourable, they should be available to the Task Force for Phase I clinical studies by 1989. At about that time, the Task Force hopes to have its long-acting androgen ester available for clinical trials. Matching an LH-RH antagonist with androgen replacement in a slow-release combination is a medium-term goal of the Task Force in the hormonal approach to contraception.

The various approaches to the development of drugs with actions either on spermiogenesis or on sperm maturation should be bearing fruit within the next or the subsequent biennium. New lines of research based on cellular and molecular biological techniques overlapping with the proposed strategy on sperm surface antigens of the Task Force on Vaccines for Fertility Regulation should offer new leads in immuniza-

tion or drug interference strategies. The various chemical synthesis programmes and the collaborative studies on purified extracts of *Tripterygium wilfordii* should also be revealing if the epididymal spermatozoa are vulnerable to drug intervention.

It will be important to continue to explore efficacy of existing methods such as vasectomy and the vas occlusive procedures based on polymers.

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