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FINAL

Report of the Joint Technical and Scientific Advisory Committees and Investigator's Meeting

Project 386-0500

Contraceptive Development: Reproductive Immunology

October 7 - 8, 1986
New Delhi

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A. Introduction

The first meeting of the Joint Indo-U.S. Technical and Scientific Advisory Committees (TSAC) for the bilateral project Contraceptive Development: Reproductive Immunology (CD:RI) was held in New Delhi on October 7-8. The meeting was convened in conjunction with the Inaugural Symposium of the National Institute of Immunology (NII).

Two joint TSAC meetings were held, the first from 9:00-10:30 am on October 7 and the second from 3:00-5:00pm on October 8. Between the two meetings, the first Investigators' Meeting was convened for Indian and U.S. scientist working on the CD:RI project. This report will summarize the two joint TSAC meetings (items B and C) and the Investigator's Meeting (item D).

B. First Joint TSAC Meeting (OCtober 7)

The following people participated:

India

Dr. S. Ramachandran, Secretary, and Department of Biotechnology (DBT)
Mr. Chaturvedi, Financial Administrator to the Department of Science and Technology (DST) and DBT
Dr. B. Saxena, Deputy Director, Indian Council of Medical Research (ICMR)
Dr. G. P. Talwar, Director, NII
Dr. N.C. Sharma, Principal Scientific Officer, NII
Dr. S.B. Bose, Administration Manager, NII

USA

Dr. N. Alexander, CONRAD/EVMS
Dr. G. Bialy, NIH
Dr. M. Harper, UTHSCSA
Mr. M. Jordan, USAID/New Delhi
Dr. N. Rose, JHU
Mr. J. Spieler, AID/Washington

Dr. Ramachandran welcomed the participants and thanked everyone for taking time to convene this first meeting of the joint TSACs. He advised the group that the Department of Biotechnology was now the Government's agency responsible for the CD:RI project; AID will be officially informed of this. He also indicated that the purpose of this first joint TSAC meeting was to deal with principles, rather that specific technical issues.

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Mr. Jordan informed the India TSAC that the Contraceptive Research and Development Project (CONRAD) at Eastern Virginia Medical School, Norfolk, would be assuming the executing agency role for the CD:RI project in place of PARFR. A formal notification will be issued shortly.

Dr. Ramachandran expressed the view that this project showed itself to be a very desirable way to collaborate and that the dollar funds available were very useful.

There was a consensus among all participants that the project was progressing very well as evidenced, in part, by the expenditure of about 60% of the budget during the first full year of operation. In addition to purchasing about $300,000 in equipment and supplies, four investigators have already participated in scientific exchanges in U. S. collaborating labs, one investigator is currently in the U.S. and two more will soon begin work in U.S. labs. Furthermore, U.S. scientists have already visited the NII for research and technology transfer projects, e.g. Dr. Sacco for zona pellucida research and several investigators for a workshop on embryo transfer

Mr. Spieler explained that USAID requires an interim project evaluation and the TSAC and Investigators' Meeting would serve this purpose. As part of the evaluation, findings and conclusions (lessons learned), recommendations and action required need to be detailed. Mr. Jordan expressed the opinion that one major lesson learned to date is that a U.S.-based intermediary acting as executor of dollar project funds appears to be an efficient and effective mechanism for project administration. Dr. Talwar complimented the two project administrators (Mrs. Krier-Morrow at Northwestern University and Mr. Bose at NII) for their competent and efficient handling of project activities. He also expressed the opinion that good team work on the part of NII, AID and Northwestern University was the key to the projects success.

The discussion turned to some specific issues related to the language of the Agreement signed between the Department of Economic Affairs (DEA) and USAID. There was general agreement that as much flexibility as possible should be retained to ensure that the project can function effectively in meetings its objectives. In this respect, it was agreed that although this first CD:RI project Agreement was for activities conducted by the NII, some limited project funds could be used at the discretion of the two TSACs for support of research conducted in other institutions in India, e.g. supplying some reagents to Dr. Sehgal at the PGI, Chandigarh, for her collaborative research with Baylor University on zona pellucida antigens.

Mr. Jordan indicated that USAID is prepared to request from AID Washington funds to extend and, perhaps, expand Indo-U.S. collaborative research in reproductive immunology by up to $1 million per year for three years. Dr. Ramachandran expressed the view that a continuation of research in this area should be implemented as a revision of the current Agreement with the NII. Should USAID wish to support reproductive immunology in other Indian institutions the NII CD:RI project could act as a funnel or umbrella project which would be more efficient than preparing separate agreements for each collaborating institution and, perhaps, involving more than one governmental department or ministry. There was some question as to how an umbrella project would function, e.g., would other institutions have to undertake collaborative projects with the NII, and did DBT forsee other institutions or investigators not wanting to receive funds through NII?

Mr. Spieler raised the issue of whether the STI (Reagan-Gandhi) projects in reproductive immunology could be brought under, or funded through, an expanded CD:RI project. Dr. Ramachandran gave reasons why this may or may not be advisable and said that he would give it further thought.

Dr. Talwar and Mr. Spieler clarified the purpose of the current CD:RI project. The project was developed to support research and scientific exchanges/training, and the purchase of supplies and equipment, to further research efforts aimed at developing contraceptive vaccines based on sperm and ovum antigens and, perhaps, some hormone antigens. In addition, technology transfer activities related to birth control vaccine biotechnology are to be supported. All members of both TSACs were provided with copies of the project Agreement. The TSACs were reminded that none of the USAID project fund could be used to support work on post-fertilization methods.

C. <u>Second Joint TSAC Meeting (October 8)</u>

The joint TSAC were reconvened on October 8, following the Investigators' Meeting (see item D below). The primary purpose of the second meeting was to share thoughts about the CD:RI project based upon what transpired over the previous two days. In addition, recommendations had to be made concerning committing the balance of the current project's budget (about $400,000). Also, the planning of any follow-on projects needed to begin.

Prior to beginning the second joint TSACs meeting, the U.S. TSAC members met to review the previous two days and to formulate its recommendations. Mr. Spieler verbally presented the recommendations with clarifications provided by other U.S. TSAC members as appropriate.

The following represents a summary of the U.S. TSAC recommendations and the discussion that resulted from the recommendations:

1. General Recommendations and Discussions

   a. In general, animal research should be conducted in lower species prior to beginning studies in primates.

   b. Brief, semi-annual reports should be prepared by the NII and shared with the joint TSACs.

   c. Equipment and supplies purchased with project funds should be retained at the NII. The equipment purchased should be for the use of the entire Institute and for other activities beside the CD:RI project.

   d. Investments in major pieces of equipment should be considered a priority within the equipment budget. Whenever possible only equipment that can be serviced in India should be purchased. The purchase of major equipment should include essential spare parts, installation and training.

   e. The India TSAC should nominate one or more scientific advisors, in addition to Dr. Badri Saxena, to act as a peer review mechanism for NII subprojects and activities to be supported under the CD:RI project. Dr. Talwar described the current NII Scientific Advisory Committee and the Research Area Panel for Reproductive Biology. It was suggested that he co-opt one or more people from the latter group to meet this recommendation.

   f. Greater attention should be paid to the design of animal studies to ensure that statistically significant, or meaningful, conclusions can be obtained. Several experiments had been conducted on too few animals to draw any meaningful conclusions. At an appropriate time, training in biostatistics may be appropriate. Dr. Talwar indicated that the NII had already recognized this need and had recruited a biostatistician.

2. Training and Exchange of Visiting Scientists Recommendations

   a. While it is recognized that short-term exchange visits or training (2-4 months) on specific subjects may be sufficient, in general, collaborative exchanges or training for 6-12 months would be more beneficial.

   b. The work of M. Chaudhuri in protein chemistry at the Salk Institute for six months was endorsed.

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c. One of the primatologists should receive additional training in the U.S. e.g. at the Oregon Regional Primate Center in Beaverton.

d. The suggestion of Dr. Talwar that Mr. Bose, the NII Administrator, attend a short-term course on management techniques was endorsed. It was suggested that he speak with Mr. Jordan concerning identifying appropriate courses.

e. It was suggested that consideration be given to training an organic chemist in amino acid synthesis. A possible host institution would be the Southwest Foundation for Biomedical Research in San Antonio, Texas (Dr. P.N. Rao).

f. Concerning the proposed visit to the U.S. of Dr. A. Suri, the U.S. TSAC recommended that work at Eastern Virginia Medical School in Dr. Alexander's laboratory would better serve his needs and the needs of the NII than a visi to the laboratory of Dr. R. Naz, George Washington University.

g. When appropriate, an engineer or technical instrument person should receive general training in equipment maintenance and repair.

- <u>CD:RI Agreement Issues</u>

a. On an ad hoc, case-by-case basis, current project funds can be used to support Indo-U.S. collaborative projects with investigators in Indian institutions other than the NII and U.S. investigators requiring very limited funding.

b. A percentage (1-5%) of the funds under the Agreement could be used as discretionary funds by the India and U.S. TSACs to permit speedy support of CD:RI project-related activities. To draw upon these funds, held by PARFR/CONRAD, the TSACs need only notify each other and indicate what the funds will be used for.

c. The current Agreement language is interpreted to state that funds are not available to support the research-related costs, other than supplies and equipment, of collaborative projects developed after work or training in the U.S., or in addition to this work or training, at the NII. Similarly, the project can not support activities in U.S. collaborative laboratories that are not directly related to the training of an Indian scientist in that laboratory. It was recommended that future agreements should have a more flexible attitude and wording.

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4. Ranking of priorities - The ranking of priorities was based upon the scientific presentations made at the Investigators' Meeting, not on the draft proposals that were prepared following the meeting and given to Dr. Talwar at the beginning of the Second Joint TSAC meeting.

The U.S. TSAC ranked the topics below in order of priority. A score of 1-5 was given to each idea; 1 being most interesting and 5 least interesting. The purpose of the ranking was to offer suggestions in the event that sufficient funds were not available to support all of these projects. It should be noted that all of the projects were considered worthy of support, some more so than others. Also, additional comments are provided on these projects under section D of this report.

1.5 - Cell-mediated immunity (Upadhyay/Anderson)
1.5 - LDH-C_4, 5-15 AA-TT studies (Gaur/Goldberg)
1.5 - Comparative zona pellucida studies (NII/Sacco/Aitken)
1.5 - Identification of sperm surface antigens using polyvalent antibodies (Shaha/Suri/Caterrhal/Bardin)
1.5 - Identification of sperm surface antigens using infertile sperm (Shaha/Suri/Alexander)
2.25 -cDNA testes library (Shaha/Bardin)
3.0 - Cloning oFSH (Jain/Chin)
3.5 - LH receptor immunization (Anand/Saxena)

Other issues that were discussed included:

1. The current CD:RI budget should be revised to include a line item for supplies, the funds to be taken from the research and training line item. The purchase of supplies should not be debited to the equipment line item.

2. The recommendation to purchase an amino acid sequencer was endorsed. It was suggested to consider the Biosystems instrument; on behalf of the NII, Dr. Chin will investigate the cost of the instrument and spares (about $100,000) and the availability of installation and training.

3. There was some discussion about a CD:RI follow-on project. The nature of the project, as well as the participating Indian institutions and modus operandi will be developed, in early 1987. Dr. Alexander and Mr. Spieler will plan to return to India in February, perhaps with other technical advisors, to plan further the extension and, perhaps, an expansion of the CD:RI project.

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4. The U.S. TSAC did not recommend that CD:RI project funds be used to support additional training or scientific exchanges and workshops in India on embryo transfer; this was not considered directly related to the purpose of the CD:RI project.

D. Investigators' Meeting (October 7-8)

The list of participants is attached as Annex 1.

Background documents for the meeting were prepared by Mrs. Diane Krier-Morrow (PARFR) and Jeff Spieler (AID/W), and distributed to the U.S. participants, Dr. Talwar and Mr. Michael Jordan. Mr. Jordan reproduced the background documents and distributed them to the India TSAC members and the collaborating investigators from the NII.

Dr. Talwar welcomed the participants which included investigators from the NII as well as from Bangalore, Chandigarh, other institutions in New Delhi and the U.S.A. He reviewed briefly the current approaches and activities of the CD:RI project. Mr. Spieler clarified that the purpose of the meeting was to review and evaluate the previously supported and ongoing activities of the CD:RI project, to recommend further activities to be conducted with the approximate $400,000 balance of the first grant ($1 million), and to make recommendations for activities that could be supported under a continuation or expansion of the CD:RI project. Furthermore, he explained that investigators from institutions other than the NII had been invited to this meeting so that they could describe their own research which could possibly be included in an expansion of the CD:RI project.

Given that the investigators from institutions other than the NII were available on the first day of the Investigators' Meeting, Mr. Spieler suggested that these investigators briefly describe their current interests in the area of contraceptive vaccines.

Dr. A. Sheth from the Institute for Research in Reproduction (IRR), Bombay, spoke about work conducted at the IRR on inhibin, monoclonal antibodies against sperm surface antigens, early pregnancy factor and riboflavin-C carrier protein.

Dr. Rajalakshmi from the All India Institute of Medical Sciences, New Delhi, described her research on epididymal specific proteins and on anti-sperm antibodies in semen and sera from men who have had vasectomy reversal.

Dr. G. Lakshmi Kumari from the National Institute of Health and Family Welfare, (NIHFW) New Delhi, described her work on isolating estrogen and progesterone receptors from uterine cytosol of women. She is also working on peptides and, in collaboration with the Indian Council of Medical Research, on the development of immuno-diagnostic tests (EIAs for estrogen and prolactin) of Medical Research Task Force on Birth Control Vaccines.

Dr. R.P. Das, also from the NIHFW, described his research on sperm function tests in infertile men, and on monoclonal antibodies to steroid receptors on sperm in rabbits that cause inhibition and immobilization of sperm.

Dr. Brij Saxena, Cornell University Medical School, New York, was asked to describe his work on developing a vaccine against LH receptors. In this regard he has already begun some collaboration with investigators at the NII looking at antibodies to the LH receptors incorporated in silastic and implanted into animals. Work conducted in New York appears to show that primary and secondary follicles in immunized animals do not progress to ovulation although they show 30% and 40% of normal progesterone and estrogen levels, respectively.

Dr. K. Murali Dhar, University of Delhi, spoke about work on the purification of buffalo pituitary gonadotropins and structure-function relation, and on bull testes inhibin. This institutions mandate is to develop training programs and to promote self-sufficiency in research.

Dr. N.R. Moudgal, Indian Institute of Science, Bangalore, mentioned his research in FSH vaccines; this had been described in detail during the NII Inaugural Symposium.

Dr. Shobha Sehgal, Postgraduate Medical Institute, Chandigarh, described her research on zona pellucida vaccines which is being supported under the Reagan-Gandhi Science and Technology Initiative (STI). She also informed the group that her institution had undertaken all of the pre-clinical animal toxicology on the NII hCG vaccines that were currently under Phase I investigation by the NII in India.

These presentations were followed by an informal review of the work of other investigators around India who were interested in vaccines for fertility regulation. It was recommended that the NII or the ICMR (perhaps through the ICMR Newsletter) seek to catalogue all of the work in India in this field. The meeting then turned to the past, current and planned activities supported at the NII under the Indo-USAID CD:RI project.

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1. Sperm antigens

a. Exchanges/Training and Research

i. S. Upadhyay/D. Anderson (CD:RI-005)

Dr. Shakti Upadhyay visited Dr. Anderson's laboratory at Harvard University for two months in order to learn immuno-precipitation and immuno-histological techniques. This work has resulted in the submission of a collaborative proposal (see section c.i)

ii. A. Gaur/E. Goldberg (CD:RI-007)

Dr. Gaur spent two months in Dr. Goldberg's laboratory at Northwestern University to learn the RIA for LDH-C_4. Dr. Gaur was also trained in quantitating LDH-C_4 antibody titers and isolating LDH-C_4 from mouse testes. Using affinity and ion exchange chromatography he learned purification procedures for LDH-C_4. This work has resulted in continued studies on LDH-C_4 at NII as well as the submission of a collaborative proposal (see section c.ii).

iii. A. Suri/R. Naz (CD:RI 003)

Dr. Naz explained that FA-1 had been isolated from testes using affinity chromatography; FA-1 in sperm is of too low a concentration for isolation. FA-1, FA-2 and FA-3 were originally identified using monoclonal antibody, MAb-24, in an affinity column. Dr. Naz stressed that large quantities of FA-1 and SHA-1 (sperm head antigen) needed to be obtained. Since the amino acid sequence of these antigens is apparently known, a question was raised as to who Dr. Naz had planned would do the peptide synthesis?

Dr. Suri was to have joined Dr. Naz at George Washington University for additional studies on two sperm antigens, SHA-1 and FA-1; his training had been postponed twice at the request of Dr. Naz. Presentation of the data to date, including Dr. Naz's description of his work at this meeting, was not very impressive. Some collaborative research had already begun at the NII as a result of a previous CD:RI project-supported visit (four months ago) to the NII by Dr. Naz. Six monkeys were injected with SHA-1, half of the immunizations were given with the adjuvant CFA and half alum and SPLPS. No antibodies to SHA-1 were measurable. The three CFA and SHA-1 immunized

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monkeys are showing "disturbed" cycles and may be pregnant. In the alum and SHA-1 group, two are pregnant and one may be pregnant. Although there are questions concerning the amount and quality of antigen injected, no evidence was presented to suggest that continued studies would be worthwhile. A similar protocol was followed for the FA-1 immunization studies. After three months of continuous mating, in the CFA and FA-1 group, two monkeys are cycling and one has a "disturbed" cycle with an enlarged uterus (may be pregnant). In the alum and FA-1 group, one animal is pregnant and two may be pregnant. Serum and vaginal washings are available and Dr. Naz proposed that the antibody titres be measured and the effects of the antisera be studied.

The U.S. TSAC and NII investigators felt that continued collaboration on this project under the CD:RI mechanism was not wothwhile. It was felt that the needs of Dr. Suri and the NII would be better served through work in Dr. Alexander's laboratory at EVMS. This being said, the TSAC did not dismiss the possibility that SHA-1 and FA-1 could, potentially, turn out to be useful antigens.

b. Equipment and Supplies

Dr. Upadhyay ordered various pieces of equipment (see annex 2). Dr. Gaur is developing an equipment list.

c. Future Activities

i. Upadhyay/Anderson

As a continuation of their previous activities Drs. Upadhyay and Anderson propose to:

a. develop a monoclonal antibody panel for identifying lymphocytes and monocyte subsets in primates. These reagents would be useful for all immuno-pathology evaluations at NII.

b. establish baseline data on lymphocyte and monocyte populations in normal animals using endometrial and endocervical biopsies and vaginal lavages.

This study is inexpensive and well thought out. It will provide useful information and skills for continued research. The US TSAC recommended that this project be supported (see annex 3). The investigation will permit the correlation between antifertility effects observed and the local tissue environment/cell-mediated response.

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ii. Gaur/Goldberg

Priorities for research on LDH-C_4 described by Dr. Goldberg included (1) expanding fertility testing of immunized monkeys, (2) correlating serum and genital tract fluid antibody titers and (3) further investigating cell-mediated immunity.

The proposed collaborative study between Dr. Gaur and Dr. Goldberg would use the bonnet monkey as a model for LDH-C_4 immunization and infertility studies.Animals will be immunized with the 5-15 amino acid sequence of LDH-C_4. Acquisition of the LDH-C_4 or the peptide will be via Dr. Goldberg. Dr. Goldberg will also work closely with Dr. Gaur to be assured that the best experimental protocol is developed and followed (see annex 4). Animals will also be immunized with LDH-C_4-BhCG-TT; however, since U.S. funds cannot be used to support research on hCG, it is inappropriate to consider this work under the CD:RI project.

iii. Shaha, Suri and Alexander

Characterization and definition of sperm antigens as possible contraceptive agents is a reasonable approach. The objective of this study is to use serum samples from infertile patients to dissect sperm antigens by means of Western blots. Potential antigens from these studies would be be used to immunize rabbits and the resultant polyclonal sera used for further studies.

These studies would be conducted at Eastern Virginia Medical School (Dr. Alexander) since immunization with extracts from fresh gels would be required. This work will provide a strong background for Dr. Suri (through his work at EVMS). Additionally, monkeys would be immunized and mated at NII; the sera from infertile animals would also be used in studies described above (see annex 5).

iv. Shaha, Suri, Caterrhal and Bardin

This project involves mapping the sperm surface antigens. The investigators plan to develop antibodies in monkeys and, similar to the previous study, raise antibodies to the blots that have been eluted. Screening of a testicular cDNA library will be undertaken. This study represents good collaboration between strong researchers (see annex 6).

Dr. Talwar suggested that a cDNA library be established for Sertoli cells. The U.S. TSAC advisors expressed reservations, especially since it was anticipated that the mRNA in sertoli cells would probably be the same as in somatic cells. It was recommended that a whole testes cDNA library would be much more useful.

2. Ovum Antigens (Zona Pellucida)

a. Exchanges/Training and Research

i. P. Thillaikoothan/A Sacco (CD:RI-006)

Unfortunately, neither Dr. Thillaikoothan nor Dr. Sacco were able to come from Detroit for this meeting. Dr. Talwar explained that he had established a collaborative program with Dr. A. Sacco. As the result of this interaction Dr. Sacco had visited the NII and forwarded a quantity of the ZP antigen which was used for immunizing 22 bonnet monkeys, using CFA and other adjuvants. Menstrual cycle disturbance and amenorrhea occurred in all animals which exhibited high antibody titers. Histolgy was done on the ovaries of several of these animals with a common feature being lack of antral follicles and corpora lutea. During the recovery phase, when antibody titers were declining, the animals showed a transition phase with infertility accompanied by high estrogen and low progesterone levels before normal cycles returned. After an extended recovery period all animals, but one, resumed cyclicity, with some becoming pregnant.

b. Equipment and Supplies

Dr. Thillaikoothan will submit a list of supplies and equipment to be purchased for the NII before his work is completed or shortly thereafter.

c. Future Activities

In order to extend this collaboration it was decided that Dr. Thillaikoothan would spend three months in the laboratory of Dr. Sacco with the aim of learning the technology required for purification of the ZP3 antigen. Most of his work/training will be on the first stages of purification with additional purification carried out at NII. The intent will be to immunize two groups of bonnet monkeys with ZP3, one with CFA and the other with SPLPS-Alum. The nature of antibody response in the two groups will be compared, and some histological studies will be performed.

In the ensuing discussion it was brought out that Dr. J. Aitken of Edinburgh claimed that marmoset monkeys immunized with a less purified ZP antigen extracted from the prepuberal porcine ovaries were infertile without showing altered menstrual cyclicity. A suggestion was made that Dr. Thillaikoothan should consider stopping at Dr. Aitken's laboratory (on return from the U.S.) in order to obtain additional information on, and a quantity of, the ZP antigen. The long-term strategy would be to use the Sacco and Aitken ZP antigens in both bonnet and marmoset monkeys for comparative purposes. A plan to obtain and breed marmosets at NII is being developed.

The U.S. TSAC advisors felt that this project has the potential of examining the utility of ZP antigens for fertility regulation. Development of a competent team in purification of antigens will be mandatory in order to achieve success. The budget for the proposed work will have to be expanded if the visit to Dr. Aitken's laboratory is to materialize.

Dr. Talwar expressed the view that longer term work in Dr. Aitken's laboratory might be preferable to a short-term stop-over by Dr. Thillaikoothan.

In a discussion of the apparent ovarian disruption caused by the Sacco antigen in bonnet monkeys (and similar results obtained by Dr. Dunbar in rabbits and Dr. Stevens in baboons) Mr. Spieler suggested the NII consider conducting research on whether ovulation can be induced inZP immunized monkeys. Such studies would answer questions about the extent of ovarian damage, e.g., whether or not there are follicles that can be recruited and stimulated to ovulate, and whether circulating antibodies would still render the animals infertile if ovulation could be induced.

3. Technology Transfer

a. Exchanges/Training and Research

i. S. Jain/W. Chin (CD:RI-001)

Dr. S. Jain visited the laboratory of Dr. W. Chin, Joslin Diabetes Center, Boston, MA for nine months (10/85-6/86).

The specific objectives of the Dr. Jain'svisit included:

a. Preparation of cDNA and genomic DNA libraries from ovine and bovine anterior pituitary gland, using

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conventional and expression vectors; identification of cDNA and genomic clones encoding the alpha and beta subunits of LH and FSH; and determination of the structure and sequence of these DNAs.

b. Expression of these DNAs in foreign mammalian cells and lower eucaryotes to produce authentic ovine and bovine gonadotrophins.

c. Isolation of the biologically active hormones from these gonadotrophin-producing cells to be used for large scale immunization protocols.

f. Assembly of a procedures manual in molecular biology for use in India.

Efforts to clone cDNAs and genomic DNAs encoding the subunits of ovine and bovine LH and FSH were successful. Dr. Jain has received training in several aspects of molecular cloning that were not in his previous extensive molecular biology experience. Dr. Jain also worked on tissue culture techniques and use of mammalian cell lines as hosts for the growth and expression of recombinant DNAs.

ii. G. Singh/N. First (CD:RI-002)

Dr. G. Singh visited the laboratories of Dr. N. First at the University of Wisconsin in Madison for three months (10/85-1/86). The objective of the visit/training was to acquire expertise in bovine and murine *in vitro* fertilization and gamete culture techniques as they relate to immunocontraceptive development and embryo transfer. Also Dr. Singh helped to plan the workshop on embryo transfer that was held at the NII in March 1986 (see item 3.c) and to purchase necessary equipment and supplies for the workshop and follow-on research.

The following techniques were learned by Dr. Singh:

1. Recovery of embryos at different stages from superovulated mice.

2. Culture of mouse and bovine embryos.

3. Transfer of mouse embryo into oviducts and uterus.

4. Manufacture of different micro-tools for micromanipulation work on oocytes and embryos.

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5. Micro-injection into pronuclei of 1 cell mouse and bovine embryos.

6. *In vitro* maturation of bovine oocytes.

7. *In vitro* fertilization of bovine oocytes and their evaluation.

8. Embryo splitting for production of identical twins.

9. Micromanipulative methods for nuclear transfer from 2-cell, 4-cell, 8-cell embryos to recipient enucleated embryos for embryo cloning.

10. Methods for superovulation of cattle.

b. Equipment and Supplies Purchased for Technology Transfer Projects

A total of $7055 was spent on equipment and $4988.55 on supplies to permit Sr. S.K. Jain to start work at NII on his return, and $209091.92 was spent on equipment and $10633.67 on supplies for the workshop and Dr. Gupreet Singh's research (see annex 2). In general, it was agreed that funds for equipment should be spent on large, costly items which might be difficult to get from other sources. It was noted that the ultrasound apparatus was now located at All India Institute of Medical Sciences, New Delhi. It was recommended that the primary use of equipment purchased under this grant should be for activities at the NII that fall within the approved workscope.

c. Workshop on "Embryo Transfer Biotechnology"

The Workshop was held in New Delhi in the Spring of 1986. The objectives of the Workshop were to provide: (1) orientation to methods of increasing reproductive efficiency in cows, and (2) training in all aspects of embryo transfer in cattle.

A written report on the workshop was provided by Dr. W.H. Eyestone, Department of Meat and Animal Science, University of Wisconsin, who was a member of the faculty for the Workshop. He concluded that the positive attitude, dedication, and competent hard work of the staff at NII, and the Dairy Research Institute, was instrumental in the success of the workshop, and that the objective of technology transfer was achieved. Future visits of Dr. Barry Bavister (University of Wisconsin) or

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Dr. Stan Leibo (Rio Vista, Texas) to assist with other aspects of this work including embryo transfer in primates, was recommended However, the U.S. TSAC advised that this would not be appropriate for continued inclusion under the CD:RI project; Dr. Bialy expressed the opinion that Dr. Bavister's consultantship could, perhaps, be facilitated by NIH if requested by the NII. Dr. Talwar indicate that all India workshop on embryo transfer and sexing was being planned for January 1987.

d. Future Activities

i. FSH

FSH plays an important role in spermatogenesis. Various reports indicate that immunizations with FSH leads to infertility in male monkeys although this has not been unequivocally demonstrated. Thus, FSH may be a candidate antigen for the development of male antifertility vaccine. A big limitation in the exploitation of this approach is the small quantity of FSH available. If ist is demonstrated the the FSH vaccine does work, it may be necessary to clone the FSH gene to produce the hormone in abundance by recombinant DNA technology. It was, therefore, proposed by Dr. Talwar that a cDNA library from ovine pituitary be prepared and expression studies be conducted. For this purpose Dr. S.K. Jain would spend three months in Dr. W. Chin's laboratory. The specific objectives would be to:

-construct a cDNA library from ovine pituitaries

-screen the library with probes

-characterize the clones to confirm identity

-extend the clone to code for full length sequence

-subclone cDNA into expression systems.

This project, although scientifically interesting was not felt to be of highest priority at this time, since immunization against FSH does not unequivically produce a suitable antifertility effect in the hands of all investigators.

ii. cDNA Testes Library

Most of the current methods of contraception deal with the female. In males, autoimmunity against sperm develop following vasectomy, and would result from immunization with sperm.

Sperm specific antibodies react with sperm surface antigens. Isolation of such sperm specific antigens requires the availability of a good cDNA testes library. A commercially available library from Clonetech has proved unsatisfactory. It was, therefore, proposed that Dr. Jain, in collaboration with Dr. Chin, prepare a monkey testicular cDNA library in phage lambda gt- 11. However, it was felt that since other testicular libraries are available, e.g. Population Council, Dr. Alexander, and others (to be determined), the preparation of another such library may not be of high priority at this time, unless the new library was sufficiently unique.

e. Other Scientific Exchanges/Training Activities

It was suggested by Dr. Talwar, and agreed to by the U.S. TSAC, that additional exchanges were required as follows:

a. Dr. M. Chaudhuri should spend 6 months at the Salk INstitute with Dr. J. Rivier for training/work in new techniques of peptide synthesis and sequencing. Dr. Rivier has already agreed to accept Dr. Chaudhuri.

b. Mr. Bose, the NII Administrator, needs training in modern management techniques. He will consult with Michael Jordan of USAID for suggestions as to appropriate training and places.

c. One of the primatologists needs to go to a good primate center (e.g., Oregon Regional Primate Center for monkeys and/or Southwest Foundation for Biomedical Research for baboons), for training in modern techniques of care and handling of primates, and for training in appropriate surgical techniques used in primate reproduction studies (e.g. endometrial biopsy).

d. At the appropriate time, one of the organic chemists should obtain advanced training in techniques of modern synthetic chemistry for steroids and amino acids, perhaps in Dr. Rao's laboratory at the Southwest Foundation in San Antonio.

4. Other Antigens

LH Receptors - Dr. Brij Saxena of Cornell University has devised methodology for quantitative recovery of the bovine corpus luteum LH receptor. Immunization of a three baboons in New York has shown that the animals exhibited normal menstrual cycles and, upon repeated mating, were infertile.

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With support from NIH, Dr. Saxena travelled to India in December 1985 and brought with him quantities of the LH receptor for the purpose of immunizing animals (rabbits). While Dr. Saxena's data are quite intriguing, insufficient information is available at this time to make a commitment to this project. Once the data from the immunization studies in India are available, possible collaboration between Dr. Saxena (NY) and Dr. R. Anand (NII) may be considered. Current immunization of bonnet monkeys is being carried out with NII resources.

5. Supply of Reagents Necessary for Recombinant DNA Research

In order to achieve the goal of cloning, sequencing and expressing genes, the constant supply of restriction endonucleases, nitro cellulose papers, vectors, primers and other chemicals of molecular biology grade are most important. These materials particularly the enzymes, nucleotide triphosphates, etc. are very labile and must be handled and transported under proper conditions. Dr. Talwar proposed that funds (e.g., U.S. $5,000 per company) should be allocated for small, frequent purchases from the following companies so the NII will be able to send a telex to these companies whenever need for reagents for research purposes arises. This arrangement will speed up research.

Companies:

1. New England Biolabs, Boston, MA
2. Bethesda Research Laboratories, Bethesda, MD
3. New England Nuclear, Waltham, MA

This proposition was accepted by the joint TSACs. The exact mechanism is to be worked out by NII and the CONRAD project staff.

The Investigators' meeting ended at 12:30pm. Investigators with projects discussed under future research were requested to prepare a brief description of the project for transmission to the Joint TSACs which were meeting again at 3:00pm.

Annex 1

CD:RI Investigators Meeting
October 7-8, 1986
National Institute of Immunology
New Delhi

List of Participants

Name	Institute
Anis Alam	National Institute of Immunology
Nancy Alexander	CONRAD, Norfolk, VA
Rajesh K. Anand	National Institute of Immunology
Deborah Anderson	Harvard Medical School, Boston, MA
K. Arunan	National Institute of Immunology,
G. Bialy	National Institutes of Health, Bethesda
Sekhar Chakrabarti	National Institute of Immunology
Renu Chaudhry	National Institute of Immunology
Manas K. Chaudhuri	National Institute of Immunology
William Chin	Harvard Medical School, Boston, MA
R. P. Das	National Institute of Health and Family Welfare, New Delhi
K. Murali Dhar	Department of Zoology, University of Delhi
Amitabh Gaur	National Institute of Immunology
Smita Gaur	National Institute of Immunology
Erwin Goldberg	Northwestern University, Evanston, IL
Chander Lekha Gupta	National Institute of Immunology
Michael Harper	University of Texas, San Antonio, TX
Joseph Hill	Harvard Medical School, Boston, MA
Ashok K. Jain	National Institute of Immunology
S.K. Jain	National Institute of Immunology
S. Jayaraman	National Institute of Immunology
Michael Jordan	USAID/India
Pramod Khandekar	National Institute of Immunology
Jaspal S. Khillen	National Institutes of Health, Bethesda, MD
G. Lakshmi Kumari	National Institute of Health & Family Welfare, New Delhi
N.R. Moudgal	Department of Biochemistry, Indian Institute of Science, Bangalore
Rajesh K. Naz	George Washington University Medical Center, Washington, DC
Rahul Pal	National Institute of Immunology
M. Rajalakshmi	All India Institute of Medical Sciences, New Delhi
L.V. Rao	National Institute of Immunology
Noel R. Rose	Johns Hopkins UNiversity, Baltimore, MD
Soumitra Roy	National Institute of Immunology
Shobha Sehgal	Post Graduate Medical Institute, Chandigarh

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Brij Saxena	Cornell University Medical Center, New York
Chandrima Shaha	National Institute of Immunology
M.G. Sharma	National Institute of Immunology
N.C. Sharma	National Institute of Immunology
Anil Sheth	Institute for Research in Reproduction, Bombay
Mukul Singh	Cornell University Medical Center, New York
Om Singh	National Institute of Immunology
Jeff Spieler	AID, Washington
Anil K. Suri	National Institute of Immunology
Shakti Upadhyay	National Institute of Immunology

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ANNEXES 3-6

The draft protocols presented in Annexes 3-6 were prepared by the collaborating investigators, with little notice, for the purpose of discussion at the second meeting of the Joint TSACs (see page 19). The protocols will be finalized by the investigators prior to commencing the research.

Annex 3

Revised Proposal (10/8/86)

Shakti Upadhyay
NII, New Delhi

Deborah Anderson
Harvard Medical School, Boston, MA

Year 1

Objectives:

1. Develop monoclonal antibody panel for lymphocytes and monocyte subset identification in primates (rhesus, baboon, langur). These reagents will be used for this study, and for all other immunopathology evaluations performed at NII in monkeys (i.e., ovarian pathology following zona pellucida immunization).

2. Establish baseline data of lymphocyte and monocyte populations in normal, untreated animals (baboon and langur because they have straight cervices). Endometrial and endocervical biopsies and vaginal lavages will be taken throughout menstrual cycle. Also, immuno-histological analysis of vaginal lymphocytes and cervical biopsies will be performed in rhesus monkeys. Animals immunized in other studies, as well as normals, may also be investigated.

Workscope:

1. Develop monoclonal antibody panel for lymphocyte and monocyte subset identification in primates (rhesus, baboon, langur):

   a. peripheral blood leukocytes
   b. lymphoid tissue sections - thymus and spleen

2. Baseline endocervical/endometrial lymphocyte/monocyte profiles throughout menstrual cycle in normal untreated animals (baboon and langur because they have straight cervix):

   a. hormone profile to establish cyclicity
   b. biopsy animals once per cycle at different times of cycle: days 1, 7, 14, 18, 22, 26 and at 6 months

3. During the first six months

   a. five animals per species (baboon and langur) - take endocervical biopsy and endometrial biopsy transcervically with Duncan Currette.

   b. Freeze, make frozen sections, fix in acetone, apply monoclonal antibodies for lymphocyte/monocyte identification

   c. Undertake analysis of vaginal lymphocyte/monocyte populations throughout menstrual cycle (baboon, langur, rhesus)

Activities and meetings of collaborators:

February 1987

1. Analyze monoclonal specificity data with aim of writing manuscript.

2. Learn biopsy technique and obtain first biopsy specimens.

3. Perform first immunohistology on biopsy specimen and discuss problems.

August 1987

Dr. Upadhyay travel to Boston for one month to review biopsy data and learn cellular immunology techniques.

1. MIF, LAI
2. lymphokine RIA (and interferon, Il-1, Il-2)
3. lymphocyte transformation (antigen)

FERTILITY STUDIES ON LDH-C_4^- IN THE BONNET MONKEY

Principal Investigators:

G.P.Talwar D.Sc.
National Institute of Immunology,
New Delhi, India.

E.Goldberg Ph.D.
NorthWestern University,Evanston,USA

Co-Investigators:

Amitabh Gaur M.Sc.
Research Officer, NII

Ashok K. Jain M.Tech.,Ph.D.
Senior Research Officer,NII

Manas K. Chowdhury Ph.D.
Senior Research Officer,NII

Collaborative project under US Agency for International Development between
National Institute of Immunology,New Delhi, India
and
NorthWestern University, Evanston, USA

Total cost US $ 3,10,400

Duration : Two Years

LDH-C_4 immunization in bonnet monkey.

Preliminary findings indicate the cessation of fertility in monkeys immunized with LDH-C_4 given with CFA. In the present study 15 monkeys would be immunized with LDH-C_4 in CFA and 15 with CFA alone, to serve as control. The anti LDH-C_4 antibodies would be monitored by immunoassays on blood and cervical mucous samples collected every two weeks. It is also proposed that a parallel experiment be carried out at North - Western University.

Same batch of the enzyme will be used for immunization at both the centres.

Objective: To study the effect of immunisation with LDH-C_4 in bonnet monkeys.

Period of Study: Two Years

Number of Animals required: 30 (in India) female bonnet monkeys.
15 male bonnet monkeys.

Period in which immunization would be completed = 3 months

Analysis of serum and cervical mucous samples and mating start after 3 months and continue throughout the period of study.

1. Animals — 45 bonnet monkeys x 700 = 31,500

Maintenance cost @ Rs.10 per animal per day = 328,500

2. Chemicals and Supplies = 1,00,000

Total Rs. 460,000

US $ 38,300

(b) Conjugation of βhCG-TT to LDH-C_4. to study the anti-fertility effects of the conjugate.

βhCG-TT-LDH-C4 conjugates would be prepared by following standardized procedures employing either periodate or maleimide esters.

The conjugates after proper characterization would be used for immunization in bonnet monkeys in the following grouping.

1. LDH-C_4-βhCG-TT — 10 Monkeys
2. βhCG-TT — 10 Monkeys
3. Vehicle — 10 Monkeys

The antibody levels to both LDH-C_4 and hCG would be monitored in both serum and cervical mucous, throughout the period of study. The animal would be put for continuous mating after the completion of the immunization schedule.

15 Males

<u>Cost Involved</u>:

1.	Animals - 45 bonnet monkeys x 700	= 31,500
	Maintenance cost @ Rs.10/animal/day	= 3,28,500
2.	Chemicals & Supplies	= 200,000
	Total	
	Rs.	5,60,000
	US $	46,600

Research Assistant - to coordinate collaborative aspects of projects; immunoassays, enzyme preparations etc.	$ 20,000
Fringe benefits @ 20%	4,000
Supplies and animals *	8,000
Travel to primate facility	2,000
Subtotal	34,000
Indirect cost @ 44%	15,000
	49,000
Budget for Two Years $	98,000

* 20 rabbits for antiserum
  2000 mice for LDH preparations

Funding for the parallel study at NII does not include the primates portion of the experiment. Approval and support will be sought from Dr. Bialy for the conduct of the work.

Project C

SYNTHETIC CONTRACEPTIVE VACCINE BASED ON AN ANTIGENIC DOMAIN OF THE SPERM SPECIFIC ANTIGEN LACTATE DEHYDROGENASE - C_4 ISOZYME

Objective :

The project is based on the synthetic approach for the development ~~for the development~~ of Contraceptive vaccine based on an antigenic domain of the sperm specific antigen Lactate Dehydrogenase - C_4 isozyme. The antigenic determinant of LDH-C_4 is known which comprises of 5-15 amino acid sequence of LDH-C_4 molecule. The peptide sequence (5-15) will be synthesized and will be linked to a macromolecular carrier, Tetanus toxoid. The immuno-biological activity of this synthetic peptide conjugated to tetanus toxoid will be tested in Bonnet monkeys.

Methodology :

The peptide LDH-C_4 (5-15) will be synthesized according to Merrifield's Solid phase method by elongating the sequence by stepwise addition of respective protected L-amino acids on to the solid support. The peptide will be finally deprotected and simultaneously cleaved from the resin using trifluoromethane sulfonic acid followed by purification by gel sieving and finally on HPLC. The amino acid composition will be confirmed by amino acid analysis and the amino acid sequence of the peptide can be doubly confirmed by protein sequencer. The peptide will be linked to other carrier, tetanus toxoid by using the conventional procedure. A small amount of the peptide and the conjugate will be sent

be Dr Goldberg's Lab for doing some immunological experiments.

EXPERIMENTAL DESIGN

All monkeys used in immunization trials will be of proven fertility and will be immunized according to the following protocol.

Immunization with peptide- conjugate :

Bonnet monkeys will be immunized with the peptide conjugate using non toxic adjuvants.

1. 10 females will receive 0.5 mg of the peptide conjugate injection - 3 injections at monthly intervals. Booster if necessary will be given after 4 months.

2. 10 females will receive 1 mg of the peptide conjugate injection - 3 injections at montly intervals. Booster if necessary will be give after four months.

3. 10 females will receive injection only with adjuvant as scheduled above.

Two weeks following the final immunization, antibody response against the peptide and the carrier will be checked, by immunoassay. If ~~there are~~ significant antibody levels are generated the animals would be put for mating with males for further fertility studies where each male will mate with two females.

Duration of project (9.1.1986 to 6.31.1988)

B U D G E T

Salary of the personnel	Nil(Employee of N.I.I. The salary will come from N.I.I.)
Supplies :-	
Chemicals	$5,000.00
Peptide sequencer(micro sequencer)	$100,000.00
	$105,000.00
Cost for Animals	
45 Bonnet Monkeys $ 65 per monkey	$ 2,925.00
Cost for the maintenance of the monkey $ 1 per day for 570 days	$25,650.00
	$28,575.00
Total :	$105,000.00
	$ 2,925.00
	$ 25,575.00
	$133,500.00

APPLICATION OF INFERTILE SERA AND AND ANTI-SPERM SERA FROM THE MONKEY FOR IDENTIFICATION OF SPERM SURFACE ANTIGENS.

Principal Investigators : G.P.Talwar, Ph.D
Nancy Alexander, Ph.D

Coinvestigators : Chandrima Shaha, Ph.D
Anil K. Suri, Ph.D

Bilateral Indo US collaboration between The National Institute of Immunology, New Delhi

and CONRAD, Eastern Virginia Medical School Norfolk, VA

Sperm autoantibodies develop spontaneously in infertile patients. These antibodies are apparently not cross reactive with any of the tissues of the body as these patients are apparently healthy. In both sexes = induction of such antibodies by active immunization can be used as a potential contraceptive method. This can be done in two ways. One is to use the infertile patient sera for screening for a sperm surface antigen, the other is to use antisperm sera raised in any other species and cross reactive with human sperm to screen for a sperm surface antigen.

OBJECTIVE : The proposed project will attempt to characterise some sperm membrane antigens by using two approaches. Antisperm antisera raised in monkeys will be used as well as sera from infertile patients.

1) The use of infertile sera from patients in dissecting out sperm surface antigens.

2) To use antisperm antisera raised in monkeys to identify various antigens.

3) Use of these antibodies to evaluate the functional role of the antigens they recognise ... characterisation by western blot and immunocytology.

4) Efficacy studies to be done by putting monkeys in mating, therefore the antigen of interest can be recognised directly form the fertility studies.

5) Human tissues will be used for testing cross reactivity of these antibodies with other organs.

6) Possible use of testicular cDNA libraries to clone the antigen of interest.

EXPERIMENTAL DESIGN :

1) Analysis of infertile sera : Infertile sera from patients will be screened by functional assays like :

i) Agglutination
ii) Immobilization

for confirmation of presence of antisperm antibodies.

For characterisation of the size of the protein recognized by the antibody these sera will be subjected to western blot analysis to identify antigens commonly found in sera from patients with immunologically mediated infertility.

At present the New Delhi group is having a number of monkey antisera which could be evaluated by the virginia group and the infertile sera with Dr. Alexander can be started ~~with~~ to be chracterised and screened by us for use in a testicular library.

Six female bonnet monkeys will be used per group for immunization with sperm. Injection will be given with MDP. Following are the methods of immunization to be used.

Immunization with various structural components of sperm :

Group 1 and 2 : Sperms will be collected by electroejaculation from bonnet monkeys, washed and stored in liquid nirogen. Membranes will be prepared after sonication and centrifuging the sperm through a sucrose gradient. Protein estimations will be done from the membrane preparation and 100ug of protein will be injected in the monkeys using MDP as an adjuvant. Immunization schedule is given later.

Group 3 and 4 : Immunization will be done with acrosome reacted sperm. Ca Ionophore A23187 will be used to cause the acrosome reaction of the monkey sperm. This is in order to expose the inner acrosomal membrane of the sperm which plays a crucial role in the sperm oocyte fusion Sperms thus treated will then be used to immunize bonnet monkeys.

Group 5 and 6 : This group will be immunized with whole sperm. Epididymal sperm from the monkeys will be used to immunise monkeys. The sperms will be washed prior to immunization.

Immunization schedule :

First injection will be given with MDP on day 1. Second injection will be given on day 10 followed by 2 subsequent injections after 10 days each. Booster will be given after 20 days of the last injection with LBA.

Monitoring of antisperm antibody titre :

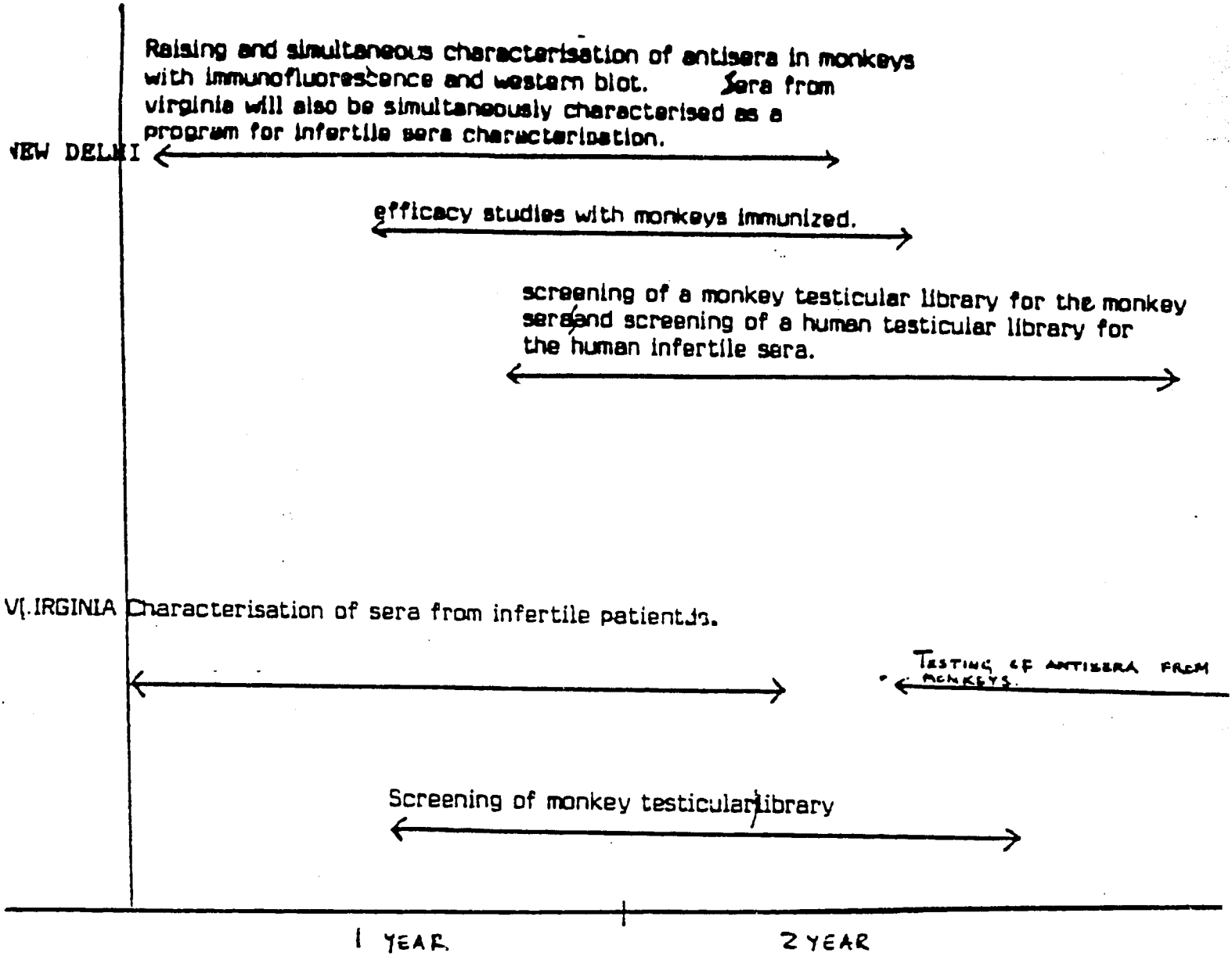
Monitoring of antisperm antibody titres will be done by Elisa and indirect immunofluorescence. This will be done to note the titres before the animals are put for mating in efficacy studies.

3) Efficacy studies :

The immunized monkeys will be set for mating once they have reached higher titres. They will be placed with males of proven fertility. Control animals will also be put for mating in the same conditions. A total of six cycles will be checked for contraceptive fertility effects.

FUTURE PLANS :

With the antigens thus identifies which plays a crucial role in the reproduction we will attempt to screen monkey testicular library with our monkey antibodies which are cross reactive with that of the human. If successfull in identifying a clone or clones they will be propagated and the fusion protein will be used to immunize rabbits for evaluation of the function of these antibodies.


Raising and simultaneous characterisation of antisera in monkeys with immunofluorescence and western blot. Sera from virginia will also be simultaneously characterised as a program for infertile sera characterisation.
NEW DELHI
efficacy studies with monkeys immunized.
screening of a monkey testicular library for the monkey sera and screening of a human testicular library for the human infertile sera.
VIRGINIA
Characterisation of sera from infertile patients.
TESTING OF ANTISERA FROM MONKEYS.
Screening of monkey testicular library
1 YEAR
2 YEAR

BUDGET

National Institute of Immunology

	IST YEAR	SECOND YEAR	
Supplies	$ 15,000	$ 16,000	
Publication Cost	$ 1,000	$ 1,000	
Travelling & Training	$ 18,000	$ 5,000	
Animals	$ 10,000	$ 5,000	
Total	$ 44,000	$ 27,000	= $71,000

Virginia

Salary of Technician	$ 18,000	$ 18,000	
Supplies(Gels etc)	$ 5,000	$ 5,000	
Animals(Rabbit sera)	$ 8,000	$ 5,000	
Total	$ 31,000	$ 28,000	= $ 59,00

Total : $ 130,000

USE OF POLYVALENT ANTIBODIES TO IDENTIFY SPERM SURFACE ANTIGENS FOR USE IN SPERM TARGETTED IMMUNOCONTRACEPTION.

Principal Investigators : G.P.Talwar, Ph.D
C.W.Bardin, Ph.D

Coinvestigators : Chandrima Shaha, Ph.D
Anil .K.Suri, Ph.D
J.Caterrhal, Ph.D

Bilateral Indo US collaboration between The National Institute of Immunology , New Delhi

and

The Population Council, New York

Sperm antigens develop relatively late in ontogenesis, therefore they are immunologically secluded in the reproductive tract in the male and are potentially foreign to the female. Therefore, they may be categorised as autoantigens in the male and isoantigens in the female. It is also well known that infertile patients are frequently found to possess antisperm antibodies. These antibodies are directed against antigens in sperm acrosome, equator, midpiece, posterior acrosome, main tail piece and tail end piece. It is important to raise polyclonal antibodies against sperm surface antigens in order to get adequate supply of antisperm antibodies for various studies related to the sperm. It would be possible to map the entire antigenic spectrum of the sperm and to investigate the functional role of various antigens involved. In this proposal we put forward the construction of a cDNA library of the human testis by the method of Okayama and Berg. This study would not only give us some valuable lead towards the search for sperm surface autoantigens which could lead to immunocontraception but at the same time would provide a mass of valuable information on basic sperm structure and function.

OBJECTIVE :

1) Production of polyvalent antibodies from carefully prepared sperm membrane preparations.

2) Characterisation of sperm surface antigens using these antibodies and mapping the antigenic spectrum of sperm with various clones of antibodies eluted from the western blots .

3) After identification of antigens immunization with gel bands to raise more specific antibodies.

4) Screening of testicular cDNA library to select out clones secreting antigens of interest for epitope selection.

5) Rescreening of testicular cDNA library with oligonucleotide probes.

6) Selection of longest cDNA, sizing, mapping and putting into expression vectors.

7) ~~Putting~~ Construction of testicular cDNA library by the method of Okayama and Berg.

At present the National Institute of Immunology has a number of polyvalent antisera that could be used for the immunopurification and some further studies in addition to raising new ones.

PREPARATION OF ANTIGENS AND EXPERIMENTAL DESIGN.

Sperm from healthy donors will be collected and stored in liquid nitrogen by the technique of semen freezing. A membrane preparation will be made by sonicating the sperm and then centrifuging through sucrose. The membrane preparation from this will be used to inject rabbits with different adjuvants.

1. With complete Freund's adjuvant
2. With MDP (Muramyl dipeptide)

CHARACTERISATION OF THE ANTISERA

The antisera will be absorbed by human blood cells to absorb out antibodies against blood group antigens. They will also be absorbed with extracts from the different organs. They will then be screened with the follwing methods.

Immunolocalization

Sperms in suspension, air dried smears and methanol fixed sperm will be used to study the localization of various antigens by using the antisera raised in rabbits with the method of indirect immunofluorescence. These will also be used to localise antigens in the testis using the immunoperoxidase method.

Sperms from different species will be checked for their cross reactivity with these antisera.

FUNCTIONAL STUDIES

Agglutination

Sperms from healthy donors will be incubated at 35°C with antisperm sera and absorbed for agglutination.

Immobilization

Inactivated serum from the immunized rabbits will be used with sperms of healthy donors to see if sperms are immobilized.

Sperm oocyte binding test

If some sera are cross reactive with lower species, functional tests will be done with that species sperms in *in vitro* binding studies.

IMMUNOPURIFICATION OF THE POLYCLONAL ANTISERA.

Running of the polyacrylamide gels : Sperm membrane extracts will be run on 10% polyacrylamide gels and then blotted on to nitrocellulose sheets.

Separation of various bands of proteins recognised by the antisera : One strip of nitrocellulose will be cut off from the nitrocellulose and then incubated with the first antibody followed by washes and incubation with the second antibody. After development of colour the bands can then be identified. The remaining nitrocellulose will then be incubated with the first antibody. After this incubation. the previously stained strip will be aligned with the remaining of the nitrocellulose and the areas representing different bands will then be cut off. Antibodies will then be eluted out of the strips by incubating them with glycine HCl at pH 2.8. They will then be neutralised to the neutral pH.

FUNCTIONAL STUDIES WITH THESE SUBCLONES.

The various clones of these antibodies thus obtained will either be tested singly or in mixtures for our functional tests mentioned earlier. Each band may represent one or several clones of the antibodies. Indirect immunofluorescnce technique will be used to localise the various antigens recognised by the several bands. Now we can map the antigenic spectrum of the sperm and identify on the gels which of the bands are most useful.

RUNNING OF SDS GELS

Bands of protein which correlate with the important proteins recognised by our immunopurification experiment will be eluted out and then SDS will be extraccted out. The bands will then be used to immunise rabbits. Some of the proteins may be carboxymethylated in order to raise non conformational antibodies which are easier to use in screening a gene library.

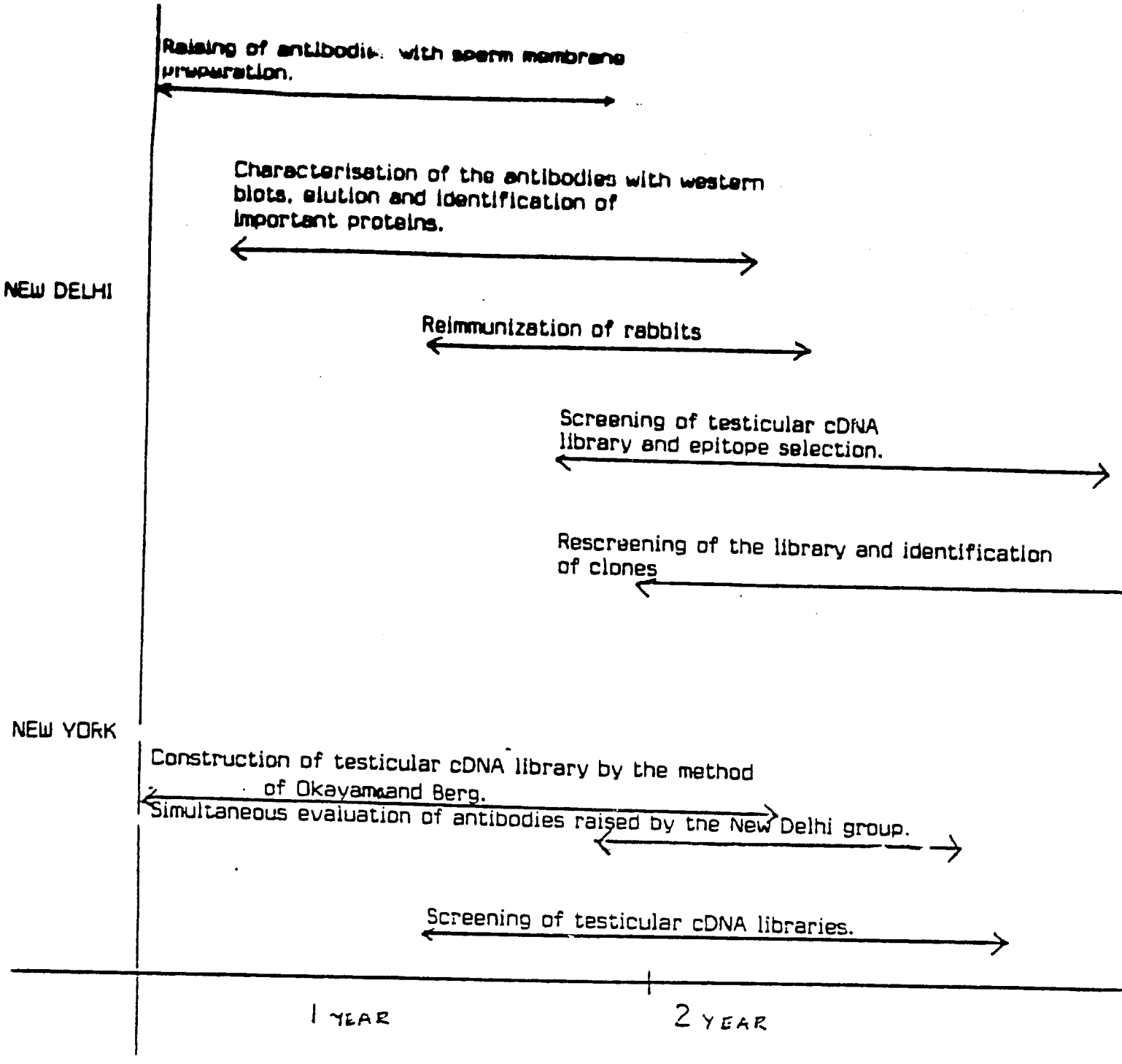
EPITOPE SELECTION

The polyclonal antisera thus raised will be verified by functional stage and then used to screen a human testicular cDNA library. The positive clones found will be subjected to primary, secondary and tertiary screening. After a clone shows 100% positive plaques the nitrocellulose with the antigen will be incubated with the antibody which is polyclonal. Each of the nitrocellulose will represent different antigens coded by the different genes. Now we can get a large supply of antibodies which can then be purified and used for various tests. The DNA from these plaques can also be purified to make oligonucleotide probes. These will be used to rescreen the testicular library in order to identify those clones that are not in the reading frame.

Longest cDNA can now be sized, sequenced, mapped and put into expression vectors. The fusion proteins can be used for active immunization studies after a few rabbits have been used to raise the sera and functionally tested.

Synthesis of a human testicular cDNA library by the method of Okayama and Berg (Moll. Cell Biol. 2, 161,1982)

construction of a testicular cDNA library will be undertaken by using the method of Okayama and Berg which enables one to get longer cDNAs. These cDNAs will then be put into chinese hamster ovarian cell line. The surface antigens coded by the gene will be expressed on the cell surface. Cells identified to be expressing the antigens will be subcloned grown in excess and the memrane separated from these cells will give a source of the antigen.


Raising of antibodies with sperm membrane preparation.
Characterisation of the antibodies with western blots, elution and identification of important proteins.
NEW DELHI
Reimmunization of rabbits
Screening of testicular cDNA library and epitope selection.
Rescreening of the library and identification of clones
NEW YORK
Construction of testicular cDNA library by the method of Okayama and Berg.
Simultaneous evaluation of antibodies raised by the New Delhi group.
Screening of testicular cDNA libraries.
1 YEAR
2 YEAR

BUDGET

	Ist Year	Second year	
National Institute of Immunology			
Supplies	$ 10,000	$ 12,000	
Animals	$ 10,000	$ 5,000	
Travel	$ 5,000	$ 5,000	
Publication	$ 2,000	$ 2,000	
Instrument	$ 20,000	$ 20,000	
Total	$ 47,000	$ 44,000	= $ 91,000
Population Council			
Supplies	$ 10,000	$ 10,000	
Travel	$ 5,000	$ 5,000	
Technician Salary	$ 20,000	$ 20,000	
Animals	$ 5,000		
Total	$ 40,000	$ 35,000	= $ 75,000

TOTAL : $ 166,000