

ANNUAL TECHNICAL REPORT

2003-2004



**National Institute for Research
in Reproductive Health**
Indian Council of Medical Research

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FOREWORD

I am pleased to present this annual report on the research activities of the National Institute for Research in Reproductive Health for the year 2003-2004. During the year, the Institute has further broadened its research horizons from 'reproduction' to 'reproductive health'. And it did not mean just the initiation of newer research initiatives but also the acquisition of newer skills, necessary infrastructure and realignment of the existing resources. It also called for the adventurous streak, diligence and enthusiasm. The Director and the staff of the institute have successfully met this challenge and they collectively have been able to create a research environment suited to the rigors of modern science. What pleases me more is that both young investigators as well as mid level scientists at the Institute have shown their keenness to venture into new areas such as microbicide development, stem cell research, transgenic animal studies, bioinformatics and reproductive toxicology. It speaks volumes about their scientific acumen and interest.

The initiative of the Institute to establish the country's first national level facility for primate breeding and research is also highly commendable. The initiative is being acclaimed by the scientific community not only in our country but in others also. The center will be a unique and valuable resource for high quality nonhuman primates of known pedigree, and will assist in studying the natural progression of diseases of primates, and address those research problems for which particular species of primates are best suited. This center will undoubtedly transform the face of biomedical research in India. I wish the staff and students of the institute success in their endeavors and I assure them of all possible support from the Council.

Prof. Nirmal K. Ganguly
Director General
Indian Council of Medical Research



Dr Anbumani Ramadoss, Union Minister of Health and Family Welfare, Government of India, solemnizing the Foundation Stone Laying Ceremony of the National Center for Primate Breeding and Research, at Sasunavgarh on 9th January 2005.



Dr Ambumani Ramadoss releasing the book on "National Center for Primate Breeding and Research: Vision, Challenges and Opportunities", on the occasion of Foundation Stone Laying Ceremony of National Center for Primate Breeding and Research



Members of the Scientific Advisory Committee which had met on 3rd November 2004 to review research programmes of the Institute.



Inauguration of the Symposium on Microbicides in Development and their potential for prevention of Sexually Transmitted Infections and Pregnancy by Mrs. Panabaka Lakshmi, Minister of State, Ministry of Health and Family Welfare, on 21-22 June 2004. Seen in the picture are Prof. N.K. Ganguly, Director General, I.C.M.R., Mrs. Panabaka Lakshmi, Dr Issac Malonza, Medical Officer, Department of Reproductive Health and Research, WHO, Geneva.



Inauguration of the International Conference on Men as Partners in Sexual and Reproductive Health, 28th November - 2nd December 2004, by Dr (Mrs) Vimal Mundada, State Minister of Health, Government of Maharashtra. Also seen are (R-L) Prof. N.K. Ganguly, DG, I.C.M.R., Dr. P.F.A. Van Look, Director RHR, WHO Geneva, Dr Altaf Lal, Health Attache, Embassy of United States of America.



The 15th Dr Shanta S Rao Memorial Oration was delivered by Dr Henry Gabelnick, Director, CONRAD on 29th November, 2004.



Dr Henry Gabelnick being conferred the Honorary Life Membership of the Indian Society for the Study of Reproduction and Fertility by Dr C.P. Puri, President, ISSRF.



Dr Anbumani Ramadoss, Union Minister of Health and Family Welfare, Government of India inaugurating the International Symposium on "Advances and Challenges in Reproductive Health Research in the Post Genomic Era", 9th January 2005.



Participants of the Workshop on Reproductive Health Research Methodology held between 23rd June and 2nd July 2004.



Participants of the WHO Workshop on IUCD, held on 26th October - 3rd November 2004.



Participant of the WHO Workshop on Ultrasonography, held during 6-10th December 2004.



Participants of the WHO Workshop on Cytology, held between 21st April- 2nd May 2004.

EXECUTIVE SUMMARY

The institute continued its research endeavors towards the accomplishment of a greater understanding of the physiologies and pathologies associated with reproductive events. The zeal to know more, learn newer skills and adopt different experimental approach, if required remained unsuppressed. The year also demonstrated the successful foray of our clinicians into the territories of basic research.



This has been a definite evidence of a highly conducive interactive research environment at the institute. In addition efforts made in the areas of stem cell research, microbicides, transgenics, reproductive toxicology also caught momentum this year.

In last couple of years, few novel sperm antigens i.e. AREP-27, HOX BISS 30, FASA57 have been discovered at the institute. This year these were characterized in detail for their functional relevance in sperm physiology. Attempts to investigate the potential of 80 KDa HSA (80 KDa human sperm antigen) sperm protein, as a target of immunocontraception were also made.

Studies on the role of genetic factors in predisposition to polycystic ovary syndrome, premature ovarian failure, congenital adrenal hyperplasia, oligoasthenozoospermia, nonobstructive azoospermia and osteoporosis were continued. We expect that with the enrollment of more subjects in next couple of years, conclusive data would be made available to facilitate the development of gene based predictive tests for these disorders.

Assessment of the prevalence, risk factors, clinical manifestations and development of diagnostic methods for reproductive tract infections (RTIs) including sexually transmitted infections are the other research activities of high priorities. Some new initiatives have been undertaken. A large number of subjects have been enrolled after careful screening to establish whether host genetic factors such as HLA predispose women to *Chlamydia trachomatis* infection. HIV receptors on spermatozoa, discovered first at the institute, have been characterized at molecular level. Attempts to obtain these proteins in purified form will definitely help develop modalities to prevent sexual transmission of HIV.

Strategies that lower the risk of acquiring RTI/STDs as well as pregnancies are expected to find more compliance by the potential users. This has been a premise of recently initiated research programmes directed towards

identification of novel microbicides offering the dual advantage. Studies were initiated to develop an effective gel formulation of Nisin, an antibacterial peptide showing spermicidal properties. These gel formulation tested in rabbits have shown their efficacies as spermicides. Studies are ongoing to prepare a gel formulation with faster release of Nisin. Simultaneous efforts to identify new compounds with antifertility and microbicidal properties have also shown some promising leads.

Programmes, specifically meant for addressing the reproductive health needs of adolescents, women and men have been extremely productive and have helped us to gauge their perceptions, attitudes and also to estimate their reproductive health needs. Inferences drawn from these interactive social research programmes will definitely help health care implementing agencies to delineate more effective strategies to cater to the specific health needs.

The institute also organized Hands on Workshops on Gynecologic Cytology and Colposcopy, and Ultrasound and conferences on Expanding Contraceptive Choices this year to create a platform for forging new scientific alliances and also to train the young investigators aspiring to become reproductive biologists. These interactive meetings also allowed the scientists at the institute to share their research observations with the external experts.

The year also witnessed the institute gearing itself for the most awaited moment of great pride and celebration i.e. inauguration of the country's first national level facility for Primate Breeding and Research. To honor this moment, the institute took the initiative to compile the research experiences of national and international primatologists and publish these as a book entitled "National Centre for Primate Breeding and Research: Vision, Challenges and Opportunities".

An overview of the significant research findings during the past year in major thrust areas has been presented in the following sections.

1. Fertility Regulation

The major objective of research in the area of fertility regulation is to expand contraceptive choices. The research programmes include development of improved and new technologies for fertility regulation, assessment of the safety, efficacy and acceptability of existing methods of fertility regulation and enhancing the role and responsibilities of men in reproductive health. The Institute currently has several such research projects in this area, funded by national and international organizations.

1.1 Identification and Characterization of epididymal Antigens using Neonatal Tolerization

1.1.1 Using neonatal tolerization

It has been well documented that sperm antigens play a crucial role in reproduction and that these proteins located on the sperm are mostly acquired either from the testis or the epididymis. Several approaches have been used for identification of these relevant proteins using conventional immunization with whole sperm followed by hybridoma technology results in antibodies to antigens invariably of testicular origin. In the current study, the approach of neonatal tolerization for raising monoclonal antibodies to rare or minor antigens was exploited by our laboratory. The monoclonal antibodies so generated as probes using epididymis specific antigens were identified. One of the five epididymis specific monoclonal antibodies obtained was characterized. Immunochemical characterization of the cognate protein revealed a tissue specific and cell type specific pattern.

The protein identified is developmentally expressed from day 30 onwards and is androgen regulated. Two forms of the protein exist, one that is held by ionic bond and leaches out along with 2M NaCl [peripheral] and the other which falls out with a detergent Triton X- 100 [integral]. The antibody identifies a protein of ~ 48kDa on a non reducing gel suggesting the presence of disulphide bonds. Immunogold labeling indicates that the protein is localized on the mid piece and tail region of the rat spermatozoa.

1.1.2 Using antisperm antibodies

Vasectomy results in the occlusion of testicular outflow leading to autoimmunity characterized by production of antisperm antibodies, (ASA). At puberty, when immune competence is already established, differentiating germ cells commence a new programme that leads to the formation of mature spermatozoa. During this process, an array of new surface molecules are expressed on the differentiating germ cells which do not belong to the family of those considered as 'self' by the immune system. Following the obstruction of male genital tract, spermatozoa/germ cells are no longer sequestered behind the blood-testis and blood epididymis barrier, resulting in an autoimmune response. Using a vasectomised mouse model, we have generated monoclonal antibodies which are being used as tools to identify and characterize functionally relevant and conserved proteins. One of the monoclonal antibodies D5E5 identifies a ~70kDa testis specific antigen .

The cognate antigen is expressed post-meiotically in a stage specific manner starting from elongating spermatids at stage 8 of spermiogenesis upto mature spermatozoa. Immunofluorescence (IIF) study shows that the antigen is localized on the tip of acrosome as well as principal piece of tail. The antigen is conserved across the species on rat, bull, marmoset and human spermatozoa as seen by IIF and exhibits species specific domain localization.

Its testis specificity, acrosome and tail localization, suggest that the cognate antigen is likely to play an important role in reproduction. Studies are in progress to unravel its role.

1.2 Studies with 80 kda Human Sperm Antigen and its Synthetic Peptides

An 80 kDa human sperm (80 kDa HSA) protein, responsible for inducing immunological infertility, has been identified, purified and characterized. Active immunization of male and female rats with 80 kDa resulted in infertility. The N-terminal peptide and peptides obtained by digestion of 80 kDa using endoproteinase Lys-C (Peptides 1-4) and Glu-C (peptides 5-6), did not show any homology with any other protein in the database. These peptides were synthesized and conjugated to keyhole lymphocyte haemocyanin and used to generate polyclonal antibodies in rabbits. A good immune response was observed with peptides NT, 1, 2 and 4.

When male rabbits were actively immunized with peptide NT and peptide 1, it resulted in agglutination of spermatozoa with complete loss of motility. Passive immunization of rats with 10 or 40 µg of the immunoglobulin fraction of antibodies to NT and peptide 1 resulted in inhibition of pregnancy in 45-70 per cent of the animals, respectively. In immunized rats, the epididymal spermatozoa were found to be agglutinated and antibodies were localized in the epididymis and not in the testis suggesting that the antibodies do not cross the blood testis barrier. Further studies with peptide 1 have been initiated in marmosets.

Earlier ontogenic studies demonstrated that 80 kDa appears in the testis of the developing rats from day 40 onwards. Current studies also indicate that in the epididymis the protein appears from day 40 onwards, suggesting that it may be androgen regulated.

1.3 Role of a Novel Androgen Regulated *Hox-B2* Containing Gene Expressed in the Epididymis

A partial sequence showing homology to the conserved region of *Hox-B2* was identified from a monkey epididymal library. In an attempt to identify epididymal proteins that play a functional role in sperm maturation, we identified a clone from the monkey cDNA library using monoclonal antibody raised against washed human spermatozoa with sequence homology to the conserved region of *Hox-B2*. The gene was expressed in adult rat, monkey and human epididymis and not in immature rat epididymis, suggesting that it is a conserved protein and is regulated by androgens. Both in the castrated as well as the ethane dimethane sulfonate (EDS) treated rat model, the protein expression in the epididymis disappeared and following supplementation with testosterone was restored to near normal levels. In developing rats, the protein was expressed from day 40 onwards, concomitant with the secretion of testosterone, also confirming its androgen regulation. The protein was expressed only in the epididymis with maximal expression in the cauda epididymis. Northern blot analysis revealed a single transcript in the monkey cauda epididymis. Attempts to obtain a full length sequence using 5'RACE resulted in a 900 bp upstream region, which has been cloned into the pGEM-T vector and is currently being sequenced.

1.4 Regulation of Endometrial Apoptosis

The human endometrium is characterized by cyclic appearances of proliferative and apoptotic changes in a steroid dependent manner. However, the precise role of steroid hormones associated with these changes remains unclear. In the present study, the female common marmoset (*Callithrix jacchus*) has been utilized as a model to study the endometrial changes in response to steroid hormones. Studies have been carried out in normally cycling females and ovariectomized females treated with graded doses of estradiol and progesterone. Using the TUNEL method, apoptosis was observed in the endometrial samples collected during the mid-luteal phase of the normal menstrual cycle. Expression of Bax, an apoptotic protein, was coincident with TUNEL and optimum plasma progesterone levels. In simulated cycles, the number of apoptotic and Bax positive cells was higher in the estradiol and progesterone treated group as compared to that in the estradiol alone treated or progesterone alone treated groups. Thus, programmed cell death in the endometrium of common marmosets occurs during the mid-luteal phase and is regulated by estrogen, progesterone and Bax protein.

1.5 Role of FSH Binding Inhibitor in Ovarian Pathophysiology

The FSH binding inhibitor (FSHBI) is an intraovarian peptide of molecular weight < 4 kDa, that inhibits FSH binding to granulosa cells *in vitro*. *In vivo*, the peptide induces atresia in mice and impairs fertility in marmosets. The N - terminal 8 amino acid fragment (Octapeptide, OP) of FSHBI exerts antifertility effects similar to that observed with the native peptide. In an attempt to study the expression and levels of OP/FSH BI in ovarian follicles, rabbit polyclonal antibodies were raised against OP and its titre assessed by ELISA. The antiserum was used for localization of OP/FSHBI in mouse ovarian follicles. While OP/FSHBI is expressed specifically in granulosa cells, the expression was higher in atretic follicles. However, no localization was noted in other mouse tissues such as liver, kidney, spleen, adrenal and uterus. The expression of OP/FSHBI in cystic ovarian follicles and estimation of their levels in cystic follicular fluid are currently ongoing.

1.6 Oocyte-Granulosa Cell Interaction During Early Folliculogenesis

Ovarian folliculogenesis is marked by the development of follicles from primordial to preantral and further to preovulatory follicle. The activation of dormant primordial follicles and their development to secondary (preantral) follicles is gonadotropin independent. The process of early folliculogenesis requires bidirectional communication between germ cells and somatic cells. The paracrine factors secreted by oocytes and somatic cells possibly regulate many of the events of early follicular development in mammals. However, factors responsible for stimulating follicles for differentiation and development are not well defined. This study was undertaken to delineate these factors using a mouse model. Morphology of the ovary and immunohistochemical localization of growth differentiation factor-9 (GDF-9), proliferating cell nuclear antigen (PCNA) and apoptosis were studied on days 2, 4 and 6 in neonatal ovaries.

GDF-9 was located in oocytes of primary follicles while, PCNA was expressed in granulosa cells of secondary follicles. Apoptosis, as revealed by TUNEL technique, was not observed during early folliculogenesis. These studies suggest that GDF-9 secreted by oocytes of primary follicles possibly interacts with granulosa cells to induce proliferation in these cells.

Studies are also in progress to understand the role of growth factors in regulation of early folliculogenesis.

1.7 Structure-Function Studies on Gonadotropins and their Receptors

Gonadotropins play a critical role in both male and female reproduction. They bind to specific high affinity receptors present on the plasma membrane of respective gonadal cells. Understanding the structural basis of this interaction would facilitate the designing of small molecules exhibiting gonadotropin-antagonistic activities. Our earlier studies with follicle stimulating hormone (FSH) suggest that the disulphide bonds Cys²⁸-Cys⁸² and Cys³²-Cys⁸⁴ play a very significant role in receptor binding by bridging the crucial residues from the regions 24 -36 and 78-90 of hFSH β resulting, in a configuration suitable for binding to the receptor. In case of the FSH receptor (FSHR), the extracellular domain has been demonstrated to be important for hormone binding. Ten different synthetic peptides corresponding to the probable surface oriented regions of ECD were studied for their ability to modulate FSH induced cAMP levels in an in-vitro FSH bioassay. Of these peptides 15-31, 79-89, 184-195, 216-235 and 285-300 exhibit an inhibitory effect indicating their involvement in signal transduction. Antipeptide antibodies raised to these peptides also inhibited the binding of hormone to its receptor indicating their ability to recognize the corresponding region on the receptor surface. Ability of the antipeptide antibodies to bind and neutralize the receptor function are being studied in depth.

To understand the patho-physiology of pituitary gonadal function, attempts are also being made to identify known as well as novel mutations in different exons of FSHR gene, and correlate naturally occurring mutations/polymorphisms in the FSHR gene and their significance

1.8 Acceptability and Continuation Rates of Two Monthly Injectable Contraceptive- Norethisterone Enanthate

To increase the range of contraceptive choices for couples in the reproductive age, a study has been initiated to evaluate the acceptability and continuation rate of two-monthly injectable contraceptive norethisterone enanthate (NET-EN).

This study was undertaken: (i) to assess user acceptability and continuation rates of Net-En; (ii) to evaluate the incidence of menstrual irregularities and other side effects; (iii) to assess the socio-behavioural aspects with respect to socio-economic and cultural diversity and (iv) study return of fertility in eligible women.

The main emphasis of this study is on good counseling by qualified and trained staff in an attempt to ensure better continuation rates.

The study is being conducted at 10 centers with a target of 1200 women from different parts of the country. So far 1070 women have been enrolled. The emphasis on counseling has ensured a good continuation rate. During the first 18 months of the study, 311 women completed 12 months of use. The preliminary observations show a cumulative continuation rate of 83.3 per cent and 67.1 per cent at 6 and 12 months of use, respectively.

1.9 Improving Male Involvement in Family Welfare

Male reproductive health involves encouraging a range of positive reproductive health issues and ensures one's own as also the well being of the family member's. In India, men play a dominant and many a times a decisive role in regulating women's access to reproductive health. Traditionally, they are not involved in parenting responsibility and often shy away from seeking reproductive health services in public. Under these circumstances, male participation is vital in enabling them to seek reproductive health knowledge and services for themselves and to extend support to their partners or spouses.

In an attempt to: (i) understand family planning knowledge, perception, attitude and practices of men; (ii) plan appropriate intervention strategies for enhancing male involvement; and (iii) evaluate the impact of the interventions on the reproductive health care of the family, the study was designed to include situational analysis in both control and experimental areas; intervention phase to increase awareness and knowledge regarding reproductive health issues and services in experimental area; and impact evaluation after three years in both the areas. Analysis of the impact analysis revealed:

Knowledge about correct use of contraceptive methods

The knowledge of correct use of condom had increased (14.1%) significantly in experimental area as compared to control area due to the interventions carried out in experimental area. Similarly the knowledge of correct use of withdrawal and rhythm a, significant increase to 27.6 percent and 27.5 percent significantly in the experimental area respectively as compared to the control area due to the interventions were observed.

Knowledge about dual purpose of condom use

The knowledge of condom use for dual purpose i.e. in prevention of pregnancy and infection had increased by 6.3 per cent in experimental area as compared to control area following interventions.

Inter-spouse communication regarding family welfare aspects

Inter-spouse communication with regard to obtaining family planning information increased by 5.4 per cent, permanent methods by 3.9 per cent and spacing methods by 0.6 per cent in experimental area as compared to control area. Significant increase in obtaining family planning information and permanent methods and marginal increase in spacing methods were observed in the experimental area .

Contraceptive use by couples

Overall contraceptive use had increased by one per cent in control area whereas it was 17.1 per cent in experimental area indicating a 16.1 per cent increase due to interventions.

The improvement was mainly due to increase in acceptance of condom, withdrawal and rhythm in experimental area. Female sterilization was widely accepted in control area, while condom use was higher in the experimental area.

1.10 Adolescent Reproductive Health

1.10.1 To Assess Knowledge, Attitude and Felt Need of Emergency Contraception amongst Adolescents and Youth, Health Care Providers and Family Planning Counselors in and Around Mumbai

Emergency contraceptives (ECs) have an important role in preventing unwanted pregnancies, induced abortions and abortion related mortality and morbidity. In view of this it is essential that the potential users as well as providers are aware of such methods and their timely correct use. In this study the knowledge, attitude and felt need of EC among adolescents and youth, health care providers, parents and teachers, woman's health advocates and chemists in and around Mumbai were assessed. Qualitative and quantitative information was collected from adolescents and youth (910), service providers and family welfare counselors (200), parents (173), teachers (237), women health advocates (WHA) and non-governmental organizations (NGOs) (27), and drug store (chemist shop) owners/employees (35).

The overall awareness among adolescents and youth regarding EC was just about 4.2 per cent, parents 8 per cent, teachers 12.2 per cent and service providers 48 per cent (ie, 75 per cent among Obstetricians and Gynecologists, 46.7 per cent among other medical practitioners, 40 per cent among family planning counselors and 31 per cent among nurses). Among WHAs and NGOs,

18.5 per cent reported being aware of ECs. The awareness among chemists and drug store owners was 60 per cent (68 per cent among medical and fancy store personnel and 40 per cent among medical stores personnel). However, even among those who were aware of EC, most of them did not have correct knowledge.

To prevent unwanted pregnancies, majority of the WHAs and NGOs opined that it was necessary to promote safer sex. Majority (37%) of WHAs and NGOs and more than one-third of the parents felt that EC methods should be provided through doctor's prescription. About 90 per cent of parents were of the opinion that sex education should be provided to prevent unwanted pregnancies. Six of every ten parents and more than half of WHAs and NGOs (55.6%) and chemists feared that EC may be misused and may affect woman's health adversely.

Equal proportion (46%) of adolescents and youth, and parents, one-fifth of teachers and three-fourth of service providers felt that EC should be made available to both married and unmarried persons. More than one-third of service providers and 15 per cent chemists and drug store owners reported that they had suggested the use of EC pills to those who had unprotected intercourse or contraceptive failure or had missed the use of contraceptive pills.

About 71 per cent of adolescents and youth, two-thirds of service providers, 70.9 per cent teachers, 50.3 per cent parents, and 88.9 per cent of WHAs and NGOs opined that EC should be included in the National Family Welfare Programme. Seven in ten teachers and 71.7 per cent of parents viewed that easy accessibility and availability of EC would increase the use among married and unmarried people.

Majority (85%) of adolescents and youth felt that EC information should be provided in their schools and colleges. About half of the service providers felt that various media should be utilized for disseminating information regarding EC. One-third of teachers and two-third of parents viewed that EC services should be provided through medical and paramedical personnel and information should be disseminated through media.

The study indicates that awareness of EC among various groups is low, attitude towards EC is positive and felt need of contraception is observed among various groups. All the respondents expressed their desire to know more about contraception and EC. The study underscores the pressing need to increase awareness and knowledge of EC through effective intervention programmes

such as audiovisuals (television and radio), print media (posters, pamphlets, brochures and flipcharts), help-line, hotline and internet services, so that unwanted pregnancies could be prevented.

1.10.2 Improving Reproductive Health of Adolescents: An Urban School Based Approach

The study was launched in two schools and two colleges to assess the reproductive health knowledge, attitude and problems among adolescents aged 11-19 years, from different socio-economic classes and to operationalise Adolescent Friendly Centers in those settings and to assess the uptake of these services.

Focus group discussions revealed that pornographic literature and films were accessed by these adolescents. Blue films were watched at home through CD's procured from special outlets using code words which represented Blue Print (BP - Bhaji Pala, Bharat Pakistan, Bharat Petroleum etc.). These were available at rates which adolescents could manage with pocket money. They were aware of websites which displayed blue films which they viewed in cyber cafes.

Knowledge on various aspects of reproductive health including HIV/AIDS was greater among boys than girls. Adolescents from the lower socio-economic class reported more reproductive health problems as compared to those from middle socio-economic class. Problems reported by girls were mainly dysmenorrhoea, irregular bleeding, excessive vaginal discharge with foul smell and pruritis vulvae. Boys reported itching of genitals, painful micturition and discharge from urethra .

Majority of adolescents from the lower socio-economic class sought household remedies for their problems, while some did go to municipal dispensaries. More boys than girls visited quacks. Adolescents from the middle socio-economic class went mainly to private practitioners.

About 13 per cent of unmarried girls in the 11-19 yr age group in low socio-economic class as against 7 per cent from the middle socio-economic class reported having had sexual intercourse. However, more girls from the middle socio-economic class reported non-coital sex .

Adolescent Friendly Centers were organized in one school and one college, and held twice a week for 2 hours each and evicted a positive response. Around 150 adolescents visited these centers during the working months of the school. A letter box was provided to maintain anonymity at each center so that the adolescents drop their questions. The answers to these questions were displayed on the notice board of the center. This intervention elicited a advice on good response and adolescents regularly visited the center for various medical problems, information and counseling.

Reproductive health checkup among boys aged 11-14 years helped diagnose 5 cases of single testis and these were counseled along with their parents and referred to a major hospital for treatment. Sixty six percent of boys aged between 11-15 years weighed below the 20th percentile and 73 per cent measured below the 20th percentile in height compared to that published by the National Centre for Health Statistics standards. About 1.2 per cent of boys and 19.6 per cent girls were severely anaemic (Hb < 8gms), 25.5 per cent boys and 25.8 per cent girls were moderately anaemic (Hb 8-10gm) and 73.4 per cent boys and 54.6 per cent girls had hemoglobin above 10gms. Interventions are ongoing.

Preliminary analysis and experiences from these studies suggest that adolescents have a quest for information on reproductive and sexual health issues. They are willing to approach adolescent friendly centers for various reproductive health problems; however their health seeking behaviour is poor. Many of the reproductive health problems like anemia, genital anomalies remain undiagnosed due to ignorance on the part of adolescents themselves and their parents. School health checkups which are part of our system, lack reproductive health focus which needs to be incorporated in order to reduce the disease burden of adolescents in adult life and hence a comprehensive approach with medical health check up is very essential in addition to providing IEC alone.

2. Infertility and Reproductive Disorders

Research programmes in this area are broadly aimed towards the identification of causes of female and male infertility, in an attempt to facilitate development of strategies for improved diagnosis and treatment. In the case of female infertility, focus of the studies has been to evaluate endocrinological and molecular caused of female infertility, and identification of markers for endometrial receptivity.

2.1 Studies on Genetic Aspects of Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a well-recognized inherited disorder. Its genetic basis has been postulated on the basis of evidences from familial clustering and reports of concordance in monozygotic twins. However, factors involved in its genetic predisposition and the mode of inheritance are still not clear. Evidence regarding diversities in its phenotypes and multiple organ involvement suggests a complex multi-genetic basis. Using a candidate gene approach, attempts to elucidate the genetic markers for determining predisposition to the disorder and determine the genotype-phenotype association were undertaken. Candidate genes selected were: CYP11A1, leptin, CYP17, follistatin and insulin receptor with emphasis on two genes viz. CYP11A1 and Leptin, associated with PCOS - hyperandrogenicity and obesity.

Subjects included in the study were those with documented evidence of PCOS in association with hyperandrogenicity with or without obesity. Those with evidence of thyroid, adrenal or pituitary disease were excluded. First degree relatives of index cases along with healthy controls were also screened. The entire coding sequence of both the genes viz. CYP11A1 and Leptin were analyzed for reported as well as novel mutations, by PCR-SSCP/RFLP for each of the exons followed by DNA sequencing to confirm the detected mutations. Fig. 16 shows PCR amplification of the coding sequences of CYP11A1. Some of the PCR amplified fragments were further subjected to SSCP analysis for detection of mutations / polymorphisms.

The SSCP analysis of exon 9 in 3 PCOS families along with 2 obese control families and 4 controls. So far screening has revealed variations in the exon 9 of CYP11A1 in two of the index cases and in the family members. Variations are being confirmed by DNA sequencing.

2.2 Genomic Studies in Women with Premature Ovarian Failure

Premature ovarian failure (POF) defined as ovarian failure occurring in women under the age of 40 years is being increasingly diagnosed. Several etiological factors have been postulated for early depletion of oocytes. Candidate genes or loci that have been suggested to cause familial or sporadic POF, include genes on the X chromosome (POF1, POF2, FMR1), inhibin and FOX L2. Data available, so far are based on Western population and there is a need to investigate this aspect in the Indian context.

The current study was initiated to determine the mutations/premutations in the FMR1, inhibin and FOX L2 genes in women with idiopathic POF and their

families and to select the best candidate gene for determining predisposition to POF.

Thirty patients diagnosed as having POF, based on elevated serum FSH levels (> 40 mIU/ml), and 25 regularly cycling fertile women were studied after establishing that their karyotype was normal. PCR amplification of the FMR1, inhibin, FOX L2 gene was carried out in all the index cases and in family members of 5 index subjects. Mutation analysis for FOX L2 gene carried out by RFLP revealed 738 C>T transition in 2 of the cases studied and in two family members of an index subject. The same mutation was not detected in any of the control samples. These observations suggest that FOX L2 gene mutations could be one of the factors associated with early ovarian failure.

2.3 Identification and Expression of Endometrial Functional Markers in Fertile and Infertile Primates

It has been established beyond doubt that the primate endometrium is receptive to the incoming embryo only during a specific period of the menstrual cycle termed as "implantation window" or "window of receptivity". The present study is aimed at deciphering the molecular mechanisms of endometrial receptivity in primates. Our previous investigations revealed that expression of various growth factors and cytokines are deregulated in the non-receptive endometrium, whereas they are positively modulated in presence of the embryo in animals during the mated cycle.

In an attempt to identify novel endometrial factors that may play a potential role in endometrial receptivity and implantation, differential display RT-PCR (DD-RTPCR) approach was used to screen endometria from fertile, infertile and mated bonnet monkeys. Three cDNA fragments have been cloned that show differential pattern of expression in onapristone-treated infertile animals as compared to control animals. One of these fragments, (DD2A) was found to be over-expressed in the endometria of onapristone treated infertile bonnet monkeys and was identified to be a Rab coupling protein (RCP) by *in silico* analysis. The results of *in silico* analysis were confirmed by RT PCR amplification using an upstream primer of RCP and a 3' primer from DD2. Sequence analysis of the 776 bp RTPCR product revealed 90 per cent homology to the human RCP sequence with conserved RAB binding domain. The RCP mRNA was found to be expressed maximally in the follicular phase endometria and downregulated in the luteal phase. RCP transcripts were found to be upregulated in the luteal phase after blocking progesterone actions or in cases of progesterone insufficiency. This is the first report demonstrating existence of RCP in the endometrium and its possible regulation by progesterone.

Using DD-RTPCR analysis, a differentially expressed cDNA fragment (gg1) that was up regulated in peri-implantation phase endometria from fecund animals as compared to controls was cloned and sequenced. BLAST search of this 600bp cDNA fragment revealed a 50 per cent homology to protein kinase A. Detailed characterization of this fragment and its regulation by steroid hormones is currently underway.

Male Infertility

2.4. Microdeletion of Y-Chromosome in Non Obstructive Azoospermic and Severe Oligoasthenozoospermic Males

Microdeletions of Y-chromosome are associated with disruption of spermatogenesis. Deletions of spermatogenic loci, known as Azoospermic Factor (AZF) are associated with infertility, and these deletions are transmitted to the male progeny. Chromosomal abnormalities and microdeletions of Y-chromosome contribute towards poor embryo quality and early pregnancy loss and detection of these would help in the management and counseling of couples undergoing IVF/ICSI. The frequency and type of deletions variable in ethnic and different population groups and there is a need to understand the role of these genes and their functional expression in spermatogenesis.

The current study aims to assess the frequency and type of microdeletions associated with non-obstructive azoospermia and severe asthenozoospermia, and to correlate the genotype/phenotype variations in these groups of men. Studies on the relative expression of these genes and their association with spermatogenesis will also be undertaken.

Salient observations

Sixty-nine infertile and 10 proven fertile men with a mean age of 32 years were recruited from the Institute's Male Infertility Clinic. The phenotypes of 41 subjects showed azoospermia and of 28 showed severe asthenozoospermia (<5 mill/ml). Almost 99 per cent of the subjects had primary infertility. Using 6 STS primers to amplify the PCR products, microdeletions of mixed type were seen in 6 subjects showing, a frequency rate of 8.6 per cent. Eight subjects underwent testicular biopsy. Phenotype/genotype correlation was seen in two subjects with deletions viz. AZFc showing severe oligoasthenozoospermia and AZFa showing azoospermia. Karyotype was normal in all cases included in the study and

control groups. Work is in progress to understand the expression of the genes in the germ line from the testicular tissue, standardization of *in situ* hybridization and immunohistochemistry.

2.5 Molecular Characterization of Human Sperm Progesterone Receptor

Classical steroid hormone receptors are transcription factors that act via a genomic mode of action. However, progesterone receptors (PR) on the human spermatozoa acts through a non-genomic mode of action. The present study is aimed at identifying and characterizing the membrane bound sperm PR and understanding its mechanism of action.

Using specific primers encoding the sequences of conventional PR (of DNA and hormone-binding domains) and using primers for its B isoform, products of the expected size were detected in the human sperm and testicular RNA by RT PCR. PR transcripts of the expected size (2.8 kb and 3.8 kb) were detected by Northern blotting of human testicular RNA. *In situ* hybridization in adult human testis revealed a stage specific expression of PR in the spermatogenic cells. Using monoclonal antibodies, directed against the conventional PR and its isoform (PR-B), also indicated stage specific expression of PR protein in the adult testis. Western blot analysis revealed three bands of 120, 94 and 55 kDa in the testicular extracts of which the 55 kDa band was detectable in the sperm lysates.

These results suggest that both PR transcripts and protein are expressed in the human testis and spermatogenic cells. The membrane bound PR appears to be translationally modified form of the conventional PR.

2.6 Identification and Characterization of Sperm Membrane Protein Profiles of Fertile and Subfertile Men

A human sperm membrane protein specific to fertile men and absent or poorly expressed in subfertile subjects has been identified. Monoclonal antibodies were raised against this 57 kDa Fertility Associated Sperm Antigen (FASA) and of the 18 hybrids, 3 were selected for limiting dilution based on their reactivity with sperm. Two clones 3H₄B7 and 3H₄A7 were characterized and these antibodies inhibited sperm-egg binding in a dose-dependent manner.

Screening a human epididymal cDNA library with a 57kDa antibody a 1.8 kb cDNA clone was identified. The sequence of this clone showed 98 per cent

homology with Human Chromosome 11 and 87 percent homology with Huntington protein (HP), which is known to be involved in microtubule formation, vesicle-mediated transport, cell-cell signaling.

3. Reproductive Tract Infections

Reproductive Tract Infections (RTIs) including sexually transmitted infections (STIs) and HIV/AIDS are being increasingly recognised as a serious public health problem. RTIs cause suffering for both men and women, but their consequences are far more devastating and widespread among women. Among women, RTIs often go undiagnosed and untreated, pelvic inflammatory disease, ectopic pregnancy, miscarriage, cervical cancer, and an increased risk of HIV transmission. Adolescents too are vulnerable to RTIs due to their ignorance of risk factors, inadequate accessibility to services and social powerhouses. A number of studies have been initiated to cover the epidemiological, clinical and diagnostic dimensions of RTIs.

3.1 *Chlamydia trachomatis* Infection: Diagnosis, Prevalence and Immunopathogenesis

Sexually transmitted infections (STIs) continues to represent a major public health concern worldwide, despite aggressive STI control efforts undertaken during the last several decades. A PCR has been standardized at the Institute to screen *Chlamydia trachomatis* infection and study the immunopathogens associated with this infection of the 91 subjects enrolled and tested for RTI's/STI's infections. The results revealed that the infection rate of *Chlamydia trachomatis* had decreased amongst women attending the gynecology clinic. Molecular HLA typing has been standardized to study the host factor. Of the infected pregnant women who were treated and followed up during the year, 4 had normal delivery and 1 had a cesarean section. All the babies were healthy and free of infection. In another collaborative study for phase-1 clinical trial of microbicide " 6% Cellulose sulphate", subjects were screened for common RTIs including Bacterial vaginosis, Candida and Trichomonas. Multiplex PCR was carried out using Amplicor PCR for screening of STI's like *Chlamydia trachomatis* and Neisseria gonorrhoeae infections before their enrollment to the study.

3.2 Transport of HIV through Spermatozoa: Identification and Characterization of CD4 Independent HIV Receptors on Spermatozoa

Human immunodeficiency virus (HIV) has been demonstrated to bind and enter into the spermatozoa and facilitate transmission into urogenital cells. However, spermatozoa have been reported to be devoid of the conventional CD4 receptors for HIV suggesting the presence of alternate receptors for HIV on spermatozoa. An HIV receptor/binding protein on spermatozoa, a 160 kDa sperm protein that is bound specifically by gp120 HIV envelop glycoprotein as well as cell free HIV, was identified by Western Blot Analysis.

The protein has been partially purified from human sperm extract by ion exchange chromatography on Mono Q column using fast protein liquid chromatography(FPLC) and is being further purified by chromatofocusing on Mono P column using FPLC.

To identify and isolate cDNA encoding the 160 kDa sperm protein, human testicular λZAP cDNA library is being immuno-screened using gp120 and its antibody. The positive clones will be further screened to isolate a full length cDNA clone for 160kDa sperm protein which will be then characterized and sequenced.

3.3. Reproductive Tract Infections: A Clinical and Microbiological Study in Women

Reproductive tract infections (RTIs) especially sexually transmitted infections (STIs) cause a wide spectrum of pathology in women and neonates, are a serious concern as presence of non-ulcerative STIs cause a 3-5 fold increase in the risk of HIV transmission.

Asymptomatic disease is responsible for frequent and severe long term morbidity of RTIs in women (PID/EP/infertility) and for the persistence and spread of STIs in the community.

The current study was initiated to evaluate the relationship between clinical manifestations and microbiological diagnosis of common RTIs (*bacterial vaginosis*, *candidiasis*, *trichomoniasis* and *chlamydia trachomatis*) in women at low risk for RTIs and to assess the therapeutic response using clinical and microbiological diagnosis.

Five hundred and sixty women attending the NIRRH Family Welfare clinics were screened and enrolled for participation in this study. The women were from lower middle socio-economic background, married, in monogamous relationships and majority were housewives.

Detailed clinical history, general and gynecological examinations as well as vaginal pH, Whiff test (10% KOH), wet vaginal smear, Papanicolaou smear and endocervical smear (for DFA for chlamydia trachomatis) were carried out.

The subjects were grouped as follows:

- Group A: 21 per cent of the total enrolled women with evidence of RTIs based on reported symptoms, clinical examination and bed side tests -were offered treatment at the initial visit
- Group B: 28 per cent of the total enrolled women with evidence of RTIs on basis of positive laboratory investigations were offered treatment at the subsequent visit
- Group C: 51 per cent of the total enrolled women with no evidence of RTIs by clinical or laboratory investigations (controls).

Eighty percent of the women came for their scheduled follow-up and completed the study. Thirty four women who were positive for *Chlamydia trachomatis* by DFA at the initial examination were given necessary treatment.

Male partners were treated for *chlamydia trachomatis* and *trichomonal* infections identified in the women. Standard therapy was administered as defined in the WHO guidelines.

These observations suggest that RTI infections, single or multiple, do occur in asymptomatic women and simple, easy methods for their detection need to be incorporated into the primary health care system. Also, the women and community need to be sensitized to distinguish between normal physiological, cyclical changes and abnormal symptoms which definitely need medical intervention. The other important aspect is the need to ensure compliance of therapy and recommend preventive measures to reduce transmission between partners.

3.4 Development and Evaluation of Newer Microbicides

Two proteins having spermicidal properties have been identified and purified from the hemolymph of Indian mud crab, *Scylla serrata*, and have been analysed for their antibacterial and spermicidal activities. Sequence analysis of these proteins is under progress.

3.4.1 Nisin – The Antimicrobial Peptide for the Control of Fertility and STDs/HIV

Over the past decade, there has been a rise in unwanted pregnancies and sexually transmitted infections (STIs). Besides, the emergence and spread of multi-drug resistant bacteria has led to increased morbidity and mortality. Therefore, our immediate goal was to develop a prophylactic contraceptive that will offer optimal dual protection for fertility control and protection against STIs/HIV.

Nisin, an antimicrobial peptide, which has been used as a food preservative for the past 50 years, appears very promising as a spermicidal microbicide. Our earlier studies indicated that, Nisin is an effective vaginal contraceptive in rats and rabbits. These studies were extended further and the safety of Nisin as an intravaginal contraceptive compound in both these species confirmed.

Studies have been initiated to determine the minimum effective concentration of Nisin using different formulations using different gel formulations such as carbapol NF or carbapol in combination with hydroxypropyl methylcellulose (HPMC).

3.5 Phase I: Expanded Safety and Acceptability Study of 6% Cellulose Sulfate

As a part of the multicenter study by CONRAD the current ongoing studies to i) determine and compare the effect of twice daily vaginal applications of 3.5 ml 6% CS or K-Y® Jelly for seven consecutive days on symptoms and signs of irritation of the external genitalia, cervix, and vagina, and epithelial disruption as seen on colposcopy in women when no intercourse is permitted; ii) to determine and compare the effect of twice daily vaginal applications of 3.5 ml 6% CS or K-Y® Jelly for seven consecutive days on symptoms and signs of irritation of the external genitalia, cervix, and vagina, and epithelial disruption as seen on colposcopy in women when intercourse is permitted; iii) to assess changes in vaginal health by results of wet mounts and gram stains in both study populations, women abstaining from intercourse and women engaging in intercourse, following twice daily vaginal applications of 3.5 ml 6% CS or K-Y® Jelly for seven consecutive days. iv) to assess the acceptability of twice daily vaginal applications of 3.5 ml 6% CS or K-Y® Jelly for seven consecutive days in women abstaining from intercourse and women engaging in intercourse; have been undertaken.

Sixty women completed the study (30 each in cohorts I and II). To enroll these 60 women the field staff contacted 493 women (potential subjects) of whom 104 reported to the clinics for screening. Among these, 57 were eligible for enrollment. Analysis of the study will be carried out by CONRAD as it is a multicenter, randomized double blind study with 3 participating sites.

3.6 Stigma Attached to HIV/AIDS: Implications for Health Care and Social Adjustment

A multi-centric study, coordinated by ICMR, has been conducted with the broad objective of assessing the nature of stigma, discrimination and denial to HIV/AIDS positive individuals in different settings and its affects in relation to seeking health care and social adjustment. The findings of the study will fill important gaps in the current knowledge and provide critical information for the design of strategies for overcoming the effects of HIV/AIDS related stigma.

The qualitative and quantitative research techniques that are being used to elicit responses are in-depth interviews of HIV sero-positive/AIDS patients (91); care givers at hospitals (60), at home (20), counselors (20) and interviews of respondents representing the general population (400 households). Data has been obtained from 91 HIV sero-positive persons, 91 care providers and 426 persons representing general population.

The major preliminary findings of the study indicate that 39 (43%) out of 91 persons living with HIV (PLWHIV) have not revealed their sero-positive status to any of their family members, to colleagues at workplace or in the community. The main reasons given were: fear of being thrown out of the house or the community; fear of losing the job and fear of being neglected. Fifty two (57%) PLWHIV shared their HIV status with either of their spouses, parents relatives and friends. The reactions at home included - did not believe; were shocked; got scared; stopped talking; and frequent quarrels including physical abuse. The community reacted by keeping a distance; stopped being inviting them to social function; verbal abuse; and total neglect. In the hospital/clinic setting, PLWHIVs perceived reactions, 50 per cent experienced a sympathetic attitude mostly from doctors while other respondents perceived negative reactions. The stigma related actions and reactions expressed by ward boys and ayahs were fear, anger and hate.

Eighty percent if the employed (N=45) respondents had not revealed their HIV status at workplace, the reasons reported were fear of losing their job, sarcasm and neglect. Regarding, modes of transmission of HIV, as perceived by all the 91 respondents, 48 (40 males and 8 females - 6 CSWs) felt that they had contracted the infection through high risk sexual behaviour. Thirty five female respondents, all housewives felt that they contracted the infection from their husbands and five respondents expressed that blood transfusion was the cause

of their getting infection by HIV virus. With regards to counseling, majority felt that better counseling, particularly post-test counseling, should be provided to the infected people as well as to their family members, particularly their spouses and close relatives. Other suggestions included support in terms of free medicines, government shelter homes, free education and food for their children.

3.7 Capacity Assessment of Primary Health Care System in a District to Deal with Reproductive Tract Infections

Community-based research on RTIs in India has so far concentrated on measuring prevalence among women and their health seeking behavior. Service delivery including training status and training needs of the health functionaries and the essential facilities for quality service provision perceive scant attention. This study was undertaken to collect information on the capacity of primary health care system (PHCS) to provide quality services for RTIs among women. This information would help to provide preventive, diagnostic and treatment services for RTIs at the PHC's .

The overall objectives of the study included : (i) to assess knowledge, skills and attitudes of health functionaries towards RTIs; (ii) to assess training needs and evolve modules for capacity building to deal with RTIs; (iii) to assess infrastructural facilities available for provision of RTI services; and (iv) identify and recommend provision of minimal essential facilities in the primary health care system for dealing with RTIs.

A facility survey of 12 PHC's and 36 subcenters, interviews of service providers working with these PHC's and subcenters; and 300 house hold interviews of women in reproductive age group within the selected areas have been completed. Data entry of the above survey is in progress.

3.8 Causes of Death by Verbal Autopsy

A multi-centric study, coordinated by ICMR is being conducted (i) to assess the probable cause of death in male and female population among rural and urban areas in Maharashtra and; (ii) to study the socio-economic profile of the households with deaths in urban and rural areas in Maharashtra. Six districts selected by probability proportional sampling in Maharashtra are Thane, Pune, Jalgaon, Akola, Yavatmal and Bhandara. Data collection is ongoing. The total numbers of deaths covered are 173 (Urban - 154 and rural- 19).

The study will develop a valid database on the age, sex and cause specific mortality that will help to anticipate future trends of mortality among the

groups. The findings will help the planners to formulate appropriate interventional strategies for prevention and control of diseases.

4. Menopause

As a part of the aging process, women above 45 years enter into the phase of menopause that leads to silent physical change including osteoporosis and genitor-urinary problems which are preventable to some extent. The Institute's research programmes have focused on prevention and management of postmenopausal osteoporosis; genitor-urinary dysfunction and to educate women about lifestyle changes and medications that may help lead a better quality of life after menopause.

4.1 Determination of Prevalence of Osteoporosis in Indian women by DEXA: Therapeutic Intervention in High Risk Women

The study was initiated to determine the prevalence and to develop norms for determining osteoporosis in Indian women. Bone density was measured by doing ultrasonography of the foot (calcaneum bone) and DEXA of the hip and spine. Data collection from 450 normal healthy women, between 25 to 75 years of age (100 women in each 10 year period and 50 women between 65-75 year age group), has been completed. Sixty women who were assessed as having osteoporosis were offered therapy either as HRT or bisphosphonates along with calcium and vitamin D or calcium and vitamin D, and monitored with the help of bone turnover markers and DEXA. The bone turnover and associated markers estimated were urinary CTx levels, serum concentrations of osteocalcin, vitamin D and plasma concentrations of intact parathyroid hormone (PTH).

The study revealed that across every five-year age band, the mean BMD measurements of the clinic data (in Indian women) were approximately 15 per cent lower than the USA (Hologic) values. The detection rates of osteopenia and osteoporosis by ultrasonography were lower than that observed by DEXA. Our study also showed that reduction in bone mass appeared earlier in the spine than at the hip and that there was an age-related decrease in BMD values. Interim data revealed that the urinary CTx values showed a significant correlation ($R=0.82$) with that of BMD. The levels of serum osteocalcin, vitamin D and plasma intact PTH levels were within the normal range.

Women's acceptance of treatment taken, side effects and improvement in bone density, if any, while on therapy were also studied. Individual and group counseling services regarding osteoporosis were held regularly at the "Elderly Women's Clinic". The results suggest the need to increase awareness among health care providers and women in general about menopause and also available

therapeutic measures that can be taken to improve the quality of life of elderly women.

4.2 Assessment of Prevalence of Osteoporosis in Adult Population in India

A study to: (i) establish peak bone mineral density (BMD) reference values for Indian men and women; and (ii) assess the prevalence of osteopenia and osteoporosis in the Indian population was undertaken. Hundred healthy men and 100 women, in the age group of 20 to 30 years and belonging to the higher socioeconomic group will be recruited to measure BMD reference values. To determine the prevalence of osteopenia and osteoporosis, BMD measurements will be taken on a total of 1500 subjects (750 men and 750 women) in the age range of 30 to 70+ years and from three socio economic groups i.e. higher socio-economic (HIG), middle socio-economic (MIG) and the lower socio-economic groups (LIG). Biochemical tests will be carried out in 20 per cent of the randomly selected subjects. Osteopenia and osteoporosis will be identified using the values generated in the first part of the study.

The staff was adequately trained prior to the initiation of studies. Household survey of volunteers from MIG has been completed. Subjects are being enrolled as per random table. A total of 356 cases have been enrolled till date and biochemical tests have been carried out among the randomly selected subjects.

4.3 Diagnostic Markers for Osteoporosis

Osteoporosis is considered as one of the significant and growing health issue amongst elderly population. Its etiology is multifunctional and several factors including hormonal, environmental and genetic factors have been linked with bone loss in men and women.

Measurement of bone density and assays of biochemical markers of bone turnover are generally used to assess age related changes in bone metabolism and predict osteoporosis. The broad objectives of the study are: i) to establish reference norms for the biochemical markers of bone turnover and document their utility in the management of osteoporosis; ii) to develop an in-house ELISA for Osteocalcin; iii) to assess whether peripheral levels of Osteocalcin can be used as an early predictor of osteoporosis; iv) to identify individuals at risk of developing osteoporosis due to their genetic make-up; v) to study the impact of environmental factors on bone health.

Biochemical markers of bone turn over were estimated in serum samples from 206 women in the age group 20-60 years. The preliminary data indicates a distinct change in the biochemical markers of bone turn over, reflecting the age related changes in bone metabolism. The levels of markers of bone turnover in 60 menopausal women who had hormone replacement therapy, showed a significant drop (9-12%) within the 3 months of therapy, reassessing the fact that biochemical markers are more useful in management of osteoporosis.

The diagnostic assays for markers of bone turn over are useful in the management of osteoporosis, however these are not prepared in India. Therefore study was initiated to develop an in house ELISA for Osteocalcin. Osteoclastin was extracted and isolated from bovine bones. Antisera have been raised and an in-house ELISA is being developed.

To establish normal reference for biochemical markers osteocalcin, bone specific alkaline phosphatase C-terminal telopeptide and pyridinium cross links (Pd/Dpd) of bone turnover, which are not available for Indian women, 175 women were enrolled. These women simultaneously underwent BMD measurements by DEXA. The bone turnover markers are being estimated and will be correlated with DEXA findings.

Cytokines are responsible for accelerated bone loss that follows loss of sex steroids during menopause. For studying mechanism of action of accelerated bone resorption during menopause, immunoassays have been standardized for interleukin 1, interleukin 6 and tumour necrosis factor alpha. The initial studies indicate that IL 6 levels are better indicator of bone turn over as markedly increased levels are observed in the samples from peri and postmenopausal women.

Bone mineral density is partly determined genetically and genetic factors contributing to fracture risk are used as markers for predicting osteoporosis. Polymorphisms in vitamin D receptor gene and estrogen receptor gene have been studied as genetic markers of genetic susceptibility. During the year method to isolate DNA from the blood has been standardized and protocols for five PCRs have been defined for the amplification of selected regions in VDR genes. These PCR products of 55 menopausal osteoporotic women were subjected to restriction digestion for restricted fragment length polymorphism study. The results indicated genetic factors play role in pathogenesis of osteoporosis.

Osteoporosis is not uncommon in men and a study has been initiated to assess the impact of environmental and genetic factors on bone health in men.

5 Genetic Disorders

Genetic disorders cause considerable burden on the family as well as the society. Therefore preventive genetics plays a crucial role in reproductive health. Our mandate is to prevent births of malformed babies by offering genetic counseling, genetic screening and prenatal diagnosis and secondly, to develop simple tests to detect carriers for thalassaemia and fragile X syndrome. Research areas include molecular basis of fragile X syndrome with emphasis on identification of premutation carrier females because they have a variable risk for ovarian dysfunction and also are at a high risk of producing affected offspring with fragile X syndrome (FXS).

The other projects on chromosomal basis of reproductive loss and genetic basis of heart disease have been addressed with emphasis on disability reduction and pregnancy losses. Cytomolecular techniques of T-FISH and sperm FISH and comparative genomic hybridisation (CGH) to detect subtle changes in the genome have also been established.

5.1 Mutational Analysis of SRY Gene in XY Sex Reversed Individuals

In human, the primary decision of male or female sexual development of the embryo is under the control of Y chromosome i.e. SRY gene. SRY gene analysis carried out in nine cases of 46, XY females showed SRY was positive in all, however no testis had developed but there were streak gonads. These gonads in the presence of Y chromosome are at a great risk for gonadoblastoma and therefore have to be removed. All these patients have been counseled.

5.2 Ascertainment of Chromosome 22q11.2 Microdeletion in- A FISH study

Congenital malformations of the heart are the most common of all birth defects occurring in 8 per 1000 live births. Risk factors contributing to Congenital Heart Disease (CHD) include abnormal levels of retinoic acid, rubella infection, smoking and alcohol consumption. The spectrum involves DiGeorge (DGS) velocardofacial syndrome (VCFS) and Conotruncal anomaly as well as non-syndromic cases. They are represented by a common microdeletion on chromosome 22q11.

The aim was to study the occurrence of microdeletion 22q11 in CHD and study familial inheritance. Of the total of 96 individuals included for genetic and FISH studies, routine G - banding showed no abnormalities. FISH studies carried out using 22q11 LSI probe, in one mother whose 2 children had died of CHD showed mosaic pattern of deletion 22q11 in 10% of her cells, the aborted fetus also showed deletion of chromosome 22q11 in 100% of the cells and the fetus was aborted due to heart disease.

Deletion of chromosome 22q11 is not very common in non syndromic CHD. FISH technique has proved to be more sensitive than classic cytogenetics. These results indicate that FISH can be used for antenatal diagnosis along with ultrasound.

5.3 Molecular Characterisation of Fragile X syndrome (FXS)

The fragile X syndrome, the most common cause of inherited mental retardation, results from unstable expansion of a trinucleotide (CGG) repeat in the FMRI gene. Phenotypic expansion is variable making clinical diagnosis difficult, while diagnosis by southern blotting is relatively expensive. The prevalence in Mumbai has not been studied. We have developed a simple, rapid screening test on blood smears to identify male patients with FXS. This is based on the presence of FMRP, the protein product of FMR1 gene in lymphocytes. We have tested the diagnostic value of this new technique by studying FMRP in 179 blood smears in cases of idiopathic male mental retardates. Of these 10 were confirmed to be fragile X syndrome. These were confirmed by Southern blots performed in collaboration with Dr. Thelma, University of Delhi. The number of lymphocytes labelled for FMRP was expressed as a percentage of the total lymphocytes screened. In normal FMRP expression is detected by a pink halo and in affected individuals there is a ghost halo. The predictive value was 100%, sensitivity 100%, and specificity 97.5%.

The project was extended to 18 BMC schools and 500 samples were screened of which 17 were found to be positive. Results of southern blot are awaited.

5.4 Cryptic Chromosomal Rearrangements in Couples with Repeated Spontaneous Abortions

Recurrent spontaneous abortions occur in 2 percent of all detected pregnancies. The causal factors are anatomical, endocrinological, immunological and genetic. Carriers of reciprocal translocation cause phenotypically abnormal offsprings due to the production of unbalanced gametes and when translocations occur in the subtelomeric region it becomes difficult to define. Recently FISH has

been used to analyse chromosomal aberrations especially in chromosome ends using telomeric probes.

The aim is to find out whether the unexplained causes of recurrent abortion are associated with subtelomeric rearrangements. A total of 89 couples were clinically and cytogenetically evaluated after ruling out all other causes of abortion. Giemsa banding revealed a normal karyotype in all cases. T-FISH was standardised and patients were subjected to T-FISH using 15 different chromosome mixtures (Ch 5p, 5q, 11p, 11q, 12p, 12q, 16p, 16q, etc). No abnormalities were seen in 5 patients screened. Simultaneously sperm FISH was standardised and used in 9 cases using 2 different combinations of probes X, Y + 18 and 13 & 21. Of the nine patients screened 3 abnormalities were detected i.e. no sex chromosome was seen in 40 sperm heads. In another patient there was loss of chromosome 21 in 22 sperm heads and an immature sperm head had X & Y signals. These findings suggest that aneuploidies play a role in male factor for RSA. The protocol for CGH is being standardised and DNA has been extracted and stored for the same.

6. Abortion

Complications from unsafe abortions if untreated, could lead to morbidity or death. The best way to prevent unsafe abortions is to reduce the unmet need for contraception and make safe abortion services accessible to women at an affordable cost. Post abortion counseling and follow up for contraception and morbidity reduction needs to be strengthened. The institute focuses its research activities on the study of women's knowledge, attitude and practice regarding abortions and to investigate abortion seeking behavior related to unwanted pregnancies, improving medical methods for termination of first and second trimester pregnancy.

6.1 Contraceptive Knowledge and Practices of Women Requesting Medical Termination of Pregnancy

A study to assess contraceptive knowledge, attitude and practices among abortion seekers in an urban hospital set up and to identify the determinants of unwanted or mistimed pregnancies in these women was undertaken.

Of the 700 women interviewed, 72 per cent were in the age group of 21 – 30 yrs, 2.4 per cent were unmarried and over 21 per cent had an induced abortion in the past. Majority of the women (94.5%) were in the first trimester of pregnancy while only 5.5 per cent had pregnancy of more than 12 weeks of

gestation. Sixty six percent of women reported the use of some contraceptive method to prevent the current pregnancy. Majority of the women never mentioned withdrawal as a family planning (F.P.) method but on in depth questioning the details of the method used were obtained.

Women reported the use of methods like withdrawal (36.5%), condom (34.4%), oral contraceptive pill (8%), rhythm (5.4%), Copper T (3.5%), tubal ligation (0.4%), spermicide (0.2%), a combination of more than one method like condom and withdrawal or rhythm (5.4%) or vaginal cleaning (5.1%). Among the women using some F. P. methods, 35 per cent had experienced condom break, leak, slip or withdrawal failure and hence were candidates for emergency contraception (EC). None of the women were aware of EC or the lactational amenorrhoea method.

Irregular use was more frequently observed with condom followed by oral contraceptive pill and withdrawal. Young child, infrequent coitus, if conceived then would opt for medical termination of pregnancy (MTP), past history of treatment taken for infertility were some of the main reasons for irregular use or non use of F. P. methods. No stock of oral contraceptive pill or condom, no suitable method for self, fear of side effects, family members against were some of the reasons wherein the women felt the need but could not use any method. Awareness of MTP related side effects was poor among women requesting an abortion for the first time (~10 %) as compared to repeat abortion (~35%). Incomplete / incorrect knowledge and myths about the various F.P. methods prevailed among these subjects.

The study results strongly stress the need for increased and sustained use of effective methods together with counseling regarding correct use, clearing myths, treatable side effects and failure rate of each method, so as to help clients decide and choose a suitable F.P. method.

7. New Initiatives

7.1 Stem Cell Research

Human development at its early stages has been difficult or impossible to study. Human embryonic stem cells offer insights into developmental events that cannot be studied directly in humans *in utero* or fully understood through the use of animal models. Studies have been initiated to understand renewal/differentiation of primordial germ cell (PGC) to spermatozoa in the male and oocyte in the female, to understand the role of regulatory factors in activation, differentiation and development of the PGC into a gamete which would also shed light on some reasons for unexplained infertility. Institute has initiated efforts to develop stem cell research to address some of these problems.

7.2 Reproductive and Genetic Toxicology

An advanced Center for Preclinical Reproductive and Genetic Toxicology has been established, which undertakes the following activities: (i) assessment of reproductive toxicity, genetic toxicity and teratology of new drugs/plant products/vaccines/devices developed nationally and internationally; (ii) evaluation of toxicity of environmental pollutants, agricultural pesticides, herbicides and fumigants and industrial wastes dioxins and xenoestrogens on reproductive functions; (iii) assessment of safety aspects of various assisted reproductive technologies e.g. intracytoplasmic sperm injection; (iv) development of rapid and reliable *in vitro* reproductive toxicity tests to monitor dose related effects, structure function relationships, etc.; and (v) human resource development for research. Toxicological studies for synthetic peptides, hormonal agents, DNA vaccines will be undertaken. The Center will inbibe general guidelines and Good Laboratory Practices as laid down by the authorities.

7.3 Bioinformatics

Modern laboratory techniques like whole genome sequencing and expression analysis are being established which would help generate data and information at the molecular level. The broad objectives of the facility are to: (i) identify novel proteins involved in the reproductive processes of male and female; (ii) assist assessment, management and research on reproductive tract infections, infertility, unsafe abortion, menopause and adolescent reproductive health; (iii) answer some major unanswered questions in reproductive biology using the leverage of information technology with applications in reproductive health; and (iv) impart training and provide facilities to scientists working in medical colleges and research institutes in the region.

7.4 Transgenic and Knockout Animal 'Models'

Facilities to generate transgenic and knockout animals to strengthen the ongoing and future research programmes, particularly in the area of cell biology are being established. At the Institute, transgenic and knockout animals will be used to understand how the testicular germ cells are differentiated after birth and what guides their maturation. Such studies will be helpful in gaining further insight for diagnosis and therapy for male infertility and designing novel methods of contraception.

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