

## Executive Summary

National Institute for Research in Reproductive Health (NIRRH), situated in the vicinity of a number of hospitals in central Mumbai, has been making vigorous efforts to improve the reproductive health of the people through research, education and health care services. The Institute is also a WHO Collaborating Center for Research and Training in Reproductive Health. Today the Institute has in its fold the Genetic Research Center, National Center for Preclinical Reproductive and Genetic Toxicology; ICMR Biomedical Informatics Center and National Center for Primate Breeding and Research. The institute collaborates with national and international research organizations in a global effort to promote multilateral exchange of knowledge pertaining to reproductive health as well as to incorporate research results into policy and programmes.



To meet the challenges posed by changing health scenario including emergence of HIV/AIDS and increased incidence of infertility in the face of rapidly changing life style patterns, the research agenda of the Institute has recently been expanded across the broad field of sexual and reproductive health, reflecting the needs and priorities of the people, including those of the vulnerable population. The institute believes in the life cycle approach to address the sexual and reproductive health needs of the population. In addition, it recognizes that the people should be able to have a satisfying and safe sex life, have the capacity to control their family size and have access to prevention of reproductive tract infections (RTIs) including sexually transmitted infections (STIs) and HIV infection.

The emphasis of the research programmes at the Institute is to increase contraceptive choices; prevent unsafe abortions; make pregnancy and child birth safer; detect, understand and intervene into the causes of male and female infertility and associated disorders; detect and prevent RTIs/STIs including HIV/AIDS; ensure that newborns are free of genetic disorders; and to help people lead a better quality of life after attaining menopause. Focus is also to create a supportive environment that would positively influence adolescents, and empower women. The institute is also on the forefront to realize the therapeutic potential of stem cells.

The strength of the institute lies in its faculty with diverse academic background and ability to adopt a multidisciplinary approach to address research issues of reproductive health concern. Most of the faculty is recognized as Ph.D. Guides by the University of Mumbai and currently over 70 students are pursuing their research programmes under their supervision. The faculty is also kept fully abreast with recent innovations including technological advances through training in best possible institutions through out the globe.

The Institute also realizes the importance of dissemination of information generated among various stakeholders and users. The international conferences organized by the institute, publication of newsletters and original research papers in peer reviewed journals of high impact factor are testimony of the institute's endeavors to be on the forefront of research in reproductive health as well as to keep the community abreast of its missions and goals.

A brief summary of the Institute profile and activities is given below, which is followed by a detailed report.

## 1. Expanding Contraceptive Choices

The research programmes to expand contraceptive choices include: (i) development of improved and new technologies for fertility regulation, (ii) assessment of the safety, efficacy and acceptability of existing methods of fertility regulation, and (iii) expanding contraceptive choices among men and young couples.

### 1.1 Development of improved and new technologies for fertility regulation

Various events during mammalian reproduction including gameto-genesis, fertilization, implantation and embryogenesis are regulated by hormones and factors secreted by reproductive and accessory organs. Identification and characterization of these regulatory factors could provide better understanding of the various reproductive processes and help in identifying newer targets for contraception and develop tools for infertility diagnosis.

Based on these premises, research programmes have been undertaken to identify new targets for male and female contraception. Screening testicular and/or epididymal cDNA libraries, screening with sera from immuno-infertile women, experimental induction or suppression of immune response to specific antigens and comparative global protein profiling of gametes from fertile and infertile men are some of the approaches being explored to identify novel proteins for the development of male contraceptive methods. These studies have led to the identification of sperm antigens such as: 27 kDa androgen regulated epididymal protein, 30 kDa epididymal homeobox protein, 57 kDa fertility associated sperm antigen, 70 kDa testis specific antigen and 80 kDa human sperm antigen (HSA) which have role in sperm function, sperm maturation and fertilization.

Of the sperm antigens so far identified, 80 kDa HSA has shown great potential for development of a contraceptive. Active immunization of marmosets, a primate species, with 80 kDa HSA as well as with a synthetic peptide deduced from HSA resulted in loss of fertility.

In the area of female fertility regulation, a human FSH binding inhibitor (FSHBI), isolated from the human follicular fluid, has been shown to inhibit FSH binding to granulosa cells, suppress ovulation and induce follicular atresia in rodents and nonhuman primates. An octapeptide, deduced from the FSHBI led to fertility impairment in nonhuman primates. In addition, research programmes are being conducted to identify potential targets for female contraception in the uterus, vagina and cervix using the proteomics and micro array approaches.

## 1.2 Assessment of the safety, efficacy and acceptability of existing methods of fertility regulation

The Institute has made significant contribution in the introduction of injectable and emergency contraceptives (EC) in the national program. A multicenter study coordinated and conducted by the Institute on evaluation of the effectiveness, safety, reversibility and acceptability of a two-monthly injectable contraceptive, norethisterone enanthate, involving 1209 women of diverse socio-economic status has reassured of the usefulness of the method for Indian population. In addition, when asked almost 90 per cent of the volunteers had responded affirmatively to the introduction of injectable contraceptive in the National Family Planning Programme. The safety and acceptability of this method was reassured, when several women who had completed two years of injection period desired to continue using this method as a contraceptive.

The Institute has developed IEC material for different target groups and created awareness about EC through workshops. A study was also undertaken to investigate whether the EC pill should be made available over-the-counter or the potential users should procure the same by prescription at the time of need. A randomized trial among condom users showed that both the approaches had merits and limitations. The merits of ready availability of the EC pill included its use by more women when the condom use had failed, as compared to when they had to procure through prescription. On the other hand, limitation of over-the-counter availability was that a greater percentage of women did not take pills as per the recommended schedule.

It was therefore recommended that the EC pills should be made available without prescription, with clear instructions regarding its use. The study also recommended establishing of hot lines to provide information about the method and its correct use to avert unintended pregnancies. These findings and recommendations facilitated marketing of levonorgestrel as an EC pill, and make it available without prescription. The IEC material developed by the Institute is being widely used by several organizations for prevention of unintended pregnancies and resulting abortions.

### 1.3 Interventions in urban slums for enhancing participation of men in reproductive health

Recognizing that male involvement is crucial to ensure good reproductive health, especially in Indian context, the Institute has been pursuing programmes to: (i) enhance awareness, knowledge and acceptance of existing spacing methods both by men and their partners by means of innovative IEC strategies in the community; (ii) provide support to women who wish to continue using temporary methods of contraception even after the completion of desired family size; (iii) increase awareness, knowledge and acceptance of existing barrier methods to reduce the incidence of STIs including HIV/AIDS; (iv) sensitize health care providers towards provision of quality services for men; and (v) build capacity and strengthen community based organizations and workers towards educating, motivating and counseling married couples.

These programmes have resulted in designing innovative community specific interventions that have effectively sensitized the couples and health care providers towards male involvement. The interventions have resulted in increase in knowledge and use of contraceptive methods; spousal communication with regard to various sexual and reproductive health issues; adoption of small family norm with emphasis on spacing between births and gender equality; use of condoms and acceptance of vasectomy; and health seeking behavior of both couples. The interventions have also illustrated the need to replicate the male involvement model designed by the Institute.

To widely disseminate information about enhancing partnership of men in sexual and reproductive health, an international conference on “Men as Partners in Sexual and Reproductive Health” was organized by the Institute. Specific recommendations for researchers, program managers and policy makers have been brought out in the proceedings of the conference.

## 2. Prevention of Unsafe Abortions

It is over three decades since the legalization of abortion in India, yet only about 15 to 20 per cent of the induced abortions are performed by the registered providers. Almost 50 per cent of maternal deaths amongst 15-19 years old girls result from the complications of unsafe abortions. High incidence of abortions, especially of unsafe abortions, can be brought down by reducing the high unmet need for contraception, method or use-related contraceptive failure, risky sexual behavior and by creating the awareness about contraception among young persons. The Institute has embarked upon research programs to identify the reasons for seeking abortions and particularly unsafe abortions as well as to develop effective and safe medical methods for pregnancy termination.

Studies showed that women from low socioeconomic strata who undergo abortion often rely more on traditional methods like withdrawal and rhythm, than on modern methods for their contraceptive needs. The use of male condom by this population was also quite irregular. These studies suggested the need for creating greater awareness about the side-effects of induced abortions; educating and counseling couples to opt for more effective contraceptives like IUD, oral pills, injectable and tubal sterilization rather than rely on abortion to control fertility. These studies also suggested the need for creating greater awareness about EC as a measure to avert unintended pregnancy.

The Institute has also played an important role in developing noninvasive, medical methods of pregnancy termination. The results of Phase III clinical trials show that the oral use of mifepristone (RU 486) and a prostaglandin analogue misoprostol provides a safe method for terminating early pregnancy. The method is effective in over 90 per cent of the cases, and has minimal side effects. The women who have had experience of undergoing pregnancy termination by both surgical and medical methods preferred medical method rather than surgical. These research endeavors at the Institute have contributed to the Drug Controller's approval to use RU 486 and misoprostol combination for first trimester abortion in the country.

### 3. Infertility and Reproductive Disorders

The Institute has carried out extensive research to identify the genetic causes of male and female infertility and to develop tools, based on state-of-the-art molecular techniques, for diagnosis and determination of susceptibility to the reproductive disorders and infertility. The tools can provide valuable adjuncts to clinicians for evidence based treatment and for screening the heritable disorders and prevention of their transmission through assisted reproductive techniques.

#### 3.1 Female infertility

In cases with polycystic ovary syndrome (PCOS), susceptibility genes have been identified and characterized using genotyping tools. Loci have been identified in the androgen regulating genes CYP11A1 and CYP17 that can provide the basis for determining genetic predisposition to the syndrome. Novel gene variants for CYP11A1 (Gene Bank accession number DQ358147) and Leptin (Gene Bank accession number DQ054472) have also been identified. These studies also helped define long term health risks associated with PCOS such as cardiovascular disease and Type 2 diabetes mellitus. Extensive studies have now been planned with regard to genomic profiling of the PCOS cases for assessing predisposition to the syndrome in various groups of populations including adolescent girls, defining the heritability pattern of mutations/polymorphisms, identifying markers for cardiovascular risk and Type 2 diabetes mellitus.

In addition, studies to explore the genetic basis for responsiveness to the various therapeutic strategies for ovulation induction are being undertaken. Current focus is on first line therapeutic agents such as clomiphene citrate in order to develop strategies for identifying non responders amongst women with PCOS, based on their genomic profile. The approach will help in personalizing the treatment and minimizing delays.

Similarly, in cases with premature ovarian failure (POF), familial aggregation of this condition suggests a genetic cause, which is being explored at the Institute. FMR1, inhibin and FOXL2 genes are being studied for permutations or mutations in women with POF and their families. A mutation in FOXL2 has been identified in some subjects and their female family members. This lead could provide a genetic tool for early detection of susceptibility to the disorder, and help in better counseling of the susceptible cases. Counseling of these women for early completion of families or cryopreservation of their ova prior to ovarian failure can help in fulfillment of their reproductive life. In the case of congenital adrenal hyperplasia, another cause of reproductive dysfunction, DNA analysis of the entire CYP21 gene revealed five of the 19 mutations commonly present in our population.

Studies to elucidate the mechanism of uterine receptivity have demonstrated suboptimal expression of integrins, interleukin-1 and 6, leukemia inhibitory factor and transforming growth factor, though the peripheral levels of steroids remained unaltered. Similarly, in cases with endometriosis research endeavors are to identify differentially expressed proteins or factors in the endometrium as well as in serum, which might contribute to the genesis of the disease. Efforts are also to develop these markers as screening tools for endometriosis.

### 3.2 Male infertility

A clinic to address the problems of infertile males has been set up at the Institute to facilitate research and elucidate the genetic and chromosomal abnormalities in nonobstructive azoospermia and severe oligoastheno-zoospermia cases. A frequency of 4.3 percent of Y chromosome microdeletions and 5.5 per cent of abnormal karyotype (Klinefelter's mosaic) has been observed in these cases. The most frequent deletions were observed in AZFb and C regions of the Y chromosome. Screening for these loci can provide valuable adjuncts to assisted reproductive techniques like ICSI and prevent transmission of the genetic abnormalities to the progeny. As both microdeletions and abnormal karyotype are associated with poor embryo quality and pregnancy loss, the screening will help in reducing these implications.

Studies undertaken to assess sperm chromatin packaging in the male partners of cases with unexplained infertility and repeated pregnancy loss showed that altered state of sperm nuclear chromatin integrity with respect to protamine concentration could be one of the male factors contributing to these conditions. Similarly, studies to

evaluate the methylation status of spermatozoa and embryo development suggested that the errors in imprinting of IGF2-H19 in the male germ cells during spermatogenesis could contribute to early pregnancy loss.

#### 4. Reproductive Tract Infections

Reproductive tract infections (RTIs) including sexually transmitted infections (STIs) and HIV/AIDS are being increasingly recognized as a serious public health problem. India has over 5.5 million estimated number of people living with HIV, and in addition around 5 per cent of the population in the reproductive age suffers from one or the other RTI/STI. RTIs cause suffering for both men and women, but their consequences are far more devastating and widespread among women. These infections often go undiagnosed and when left untreated, they lead to complications such as infertility, 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 and vulnerability to risk factors and inadequate accessibility to services.

High priority has been given at the Institute to pursue research programmes to cover the diagnostic, epidemiological and clinical dimensions of RTIs/STIs. The research programmes include: development of simple, cost-effective, sensitive, and rapid diagnostics for RTIs; estimation of population based prevalence of RTIs; assessment of primary health care system to deal with RTIs; understanding the mechanism of HIV transmission; development of microbicides; and understanding the relationship of RTIs with other disorders such as cervical cancer and infertility. The highlights of some of the research findings are given below.

##### 4.1 Development of microbicides

A comprehensive research programme has been launched to prevent STIs by topical application of microbicides. It includes: the understanding of molecular basis of interaction of pathogen with host cells and onset of infectious process; to define vaginal and cervical ecology and natural defense mechanisms of the female reproductive tract; to evaluate candidate molecules for development of topical microbicides; clinical evaluation of candidate microbicides; and to raise cohorts to participate in clinical trials.

A number of vaginal microbicides are currently undergoing preclinical evaluation to assess their efficacy, safety and acceptability. Two such molecules, namely magainin and nisin, have shown antimicrobial and spermicidal activities.

Intravaginal application of these peptides blocked conception in rabbits. Nisin has antibacterial activity, and is being investigated for its anti-HIV activity.

Since crabs are resistant to microbial infections, studies are also being pursued to isolate compounds with antimicrobial activity from the hemolymph of Indian mud crab (*Scylla serrata*). Two cationic proteins (12 kDa and 19 kDa) so far isolated have shown antimicrobial and spermicidal activities. Sequencing of the 12 kDa peptide identified a 19 amino acid peptide that showed sequence homology with cystatin, a known antimicrobial compound. This 19 amino acid protein has been synthesized at the Institute and is being further evaluated for use as a microbicide.

## 4.2 National guidelines for management of RTIs/STIs

In the Phase-1 of the National Reproductive and Child Health (RCH) program in India, STI/RTI services could not be operationalised below the district levels. Recognizing the potential of the Institute, the Government of India had called upon the Institute to develop National Guidelines for Management of RTIs/STIs that could be incorporated in the RCH-2 program.

The inputs required for framing the management guidelines for RTIs/STIs were drawn from desk research and primary data collection, which was collected through countrywide Rapid Assessment Surveys (RAS) on the representative samples of facilities selected from six zones in the country. The RAS assessed the situation on programme management at state and district levels, infrastructure, availability of equipments, supplies and drugs, availability of IEC material, record keeping and accessibility of RTI/STI services to the community. The needs and preferences of the community for RTI services in terms of place, service provider, and cost were also assessed.

The major gaps observed were inadequate coordination between HIV/AIDS and RCH programme, inadequate space for examination in privacy and confidentiality, facilities to conduct laboratory tests for RTIs, inadequate knowledge on RTI management among service providers, poor follow-up and partner management and unavailability of IEC material. Community level data indicated poor knowledge on causation and prevention of RTIs as compared to HIV/AIDS.

Simultaneously, the working groups had prepared the draft clinical management guidelines, the highlights of which included an overview of RTI/STI epidemiology; approach to comprehensive RTI/STI case management; user friendly management flowcharts including syndrome-specific partner management and management of pregnant women; effective drug regimens with single oral dosages wherever possible; issues of privacy and confidentiality, and partner management given special focus; opportunities and approach for detection of asymptomatic RTIs/STIs by screening tools; dual protection options and integration of RTIs/STIs assessment into FP services; special emphasis on RTIs in context of pregnancy; RTIs/STIs among special populations like neonates and adolescents; infection

control and universal safety precautions; procedures for simple laboratory tests which can be done at PHC level. The implementation guide for State and District level program officials is also under preparation. The developed guidelines will be presented for review in a broad-based national consultation meeting.

#### 4.3 CD4 independent transmission of HIV

The Institute was the first to demonstrate the presence of HIV binding protein on spermatozoa, which is known to lack conventional CD4 receptor. Partial amino acid sequencing and cDNA sequencing of this protein of 160 kDa has demonstrated its similarity with human mannose receptor (hMR).

Initial analysis in serodiscordant couples, where males remain HIV negative even after unprotected sex with their HIV positive spouses has suggested the possible role of sperm MR in HIV transmission. Our studies have reinforced the possibility of HIV virus utilizing different receptors in specialized cells such as sperms. This calls for exploring the strategies to prevent HIV infection by targeting both CD4 dependent as well as independent receptors.

#### 5. Adolescent Reproductive Health

India is a home for more than 350 million adolescents in the age group of 10-24 years. This period is the most crucial phase, as many key biological and psychosocial events occur that set the stage for the adult life. The health needs of this vulnerable group have not been addressed adequately; as their reproductive health needs are often misunderstood, unrecognized or underestimated.

The institute has chalked out a comprehensive research program to address reproductive health of adolescents by creating service delivery models in different settings, both rural and urban areas such as in schools and colleges and in urban health posts jointly with Municipal Corporation of Greater Mumbai. The overall objective is to create a supportive environment that would positively influence knowledge, attitude, perception, skills and behavior of adolescents by using effective IEC and counseling and meeting their health needs by providing quality reproductive and sexual health services at the Adolescent Friendly Centers. The interventions have resulted in enhanced utilization of services at the Adolescent Friendly Centers for information, counseling and medical services for a number of reproductive health problems.

The need for desensitizing the teachers in matters related to sexual and reproductive health came as another important observation. Improvement in knowledge and attitudes by 20-40 percent has demonstrated the effectiveness of IEC programs. Post intervention the centers at the urban health post got several referrals from the community and also from the health post staff reflecting that a supportive environment was being created. The demand for IEC activities/health checkup

campus has been on the rise and the interactions with various groups have a good impact in creating an adolescent friendly environment in the community as well as among health care providers. This initiative of the Institute has pointed out the need for providing more specific adolescent focused health services such as provision of information, counseling and services with quality of care.

## 6. Maternal and Child Health

It is now well recognized that all drugs and chemicals in the maternal circulation can be transferred to breast milk making the nursing an unintended recipient of these drugs. Among the major factors influencing infant's exposure to these drugs are quantity of drug transferred to breast milk and mother's capacity for metabolizing these drugs. To minimize the exposure of the nursing to the maternal drugs, transfer potential of each of these drugs must be known. Since tuberculosis occurs commonly among women in reproductive age, necessitating chronic therapy even during lactation, study has been undertaken to determine the transfer potential of anti-tuberculosis drugs from circulation to breast milk and assess the variability in individual capacity for metabolizing these drugs through pharmacogenomic analysis.

Transfer potentials of the drugs (Rifampicin, Isoniazid and Ethambutol) are being determined in terms of Milk/Plasma ratios. The drug levels are determined by HPLC in milk and plasma collected at different time intervals (1, 2, 3 and 4 hrs) following ingestion. For assessment of drug metabolizing capacity, study has been initiated with regard to the enzyme N-acetyl transferase 2 (NAT2) which metabolizes the drug Isoniazid. All known polymorphisms in the gene encoding NAT2 are being screened by PCR-RFLP.

Assessment of the NAT2 profile showed that polymorphic cases had a slower rate of metabolism of the drug as compared to non polymorphic cases. With regard to transfer of the drug to breast milk, it was found to be maximum (65%) at 3 hours following ingestion of the drug. Further analysis is in progress.

Programmes will also be initiated to address the common causes of high-risk pregnancy such as pre-eclampsia, gestational diabetes, infections during pregnancy, which contribute substantially to maternal and fetal morbidity and mortality. Research focused on understanding the physiology of cervical ripening and parturition would unravel the intricate mechanisms involved in pre-term or post-term parturition and help in developing new and more effective drugs to prevent pre-term delivery or for induction of labor.

## 7. Osteoporosis and Menopause

With the increase in life expectancy at birth and the age at the menopause remaining almost the same, the number of women living after attaining the age of

Menopause is increasing. The depletion of hormones during peri-menopausal phase is associated with variety of physiological changes, such as osteoporosis, arterial diseases and urogenital problems. These problems are to a greater extent preventable, if diagnosed early and timely intervened.

Osteoporosis is now considered an important public health issue. It is one the most common consequences of old age. Measurements of bone mineral density and biochemical markers of bone turn over are widely used parameters for diagnosis and management of osteoporosis. The markers of bone turn over are a more sensitive indicator of changes occurring in bone mass. The Institute initiated a study to develop ELISA for markers of bone turn over and an in house ELISA for osteocalcin.

The Institute has focused its research programme on (i) establishing base-line norms for the parameters such as bone density, osteocalcin, bone specific alkaline phosphatase, pyridinoline, deoxypyridinoline and N and C terminal telopeptide, used in the diagnosis of osteoporosis; (ii) developing the methods for prevention and management of postmenopausal osteoporosis as well as early onset of osteoporosis; (iii) identifying genetic factors contributing to osteoporosis; and (iv) to study the impact of environmental or life-style factors on bone health.

Studies have revealed that peak bone mineral density (BMD) at hip and spine of healthy Indian women, measured by Dual Energy X-ray Absorptiometry (DEXA), in the age group of 20 to 60 years was 10-15 per cent lower than that in age matched women in USA. About 25 per cent of young women were found osteopenic, whereas 5-10 per cent osteoporotic. The incidence of osteoporosis was higher in women as compared to men.

Another study conducted at the Institute showed beneficial effects of bisphosphonate in combination with calcium and vitamin D on BMD in osteoporotic women. This study formed the basis for proposing dietary supplementation with calcium and vitamin D to reduce the chances of early onset of osteoporosis in women.

Research programmes are being pursued to develop indigenous assays to assess bone turnover. Assays for quantitation of osteocalcin, bone specific alkaline phosphatase, pyridinoline, deoxypyridinoline and N and C terminal telopeptide levels in serum have been developed and reference norms being established for these markers in Indian population. In conjunction with the ovarian hormones, these markers can aid in early identification of women at increased risk of osteoporosis.

Studies with an objective to unravel the role of genetic factors in determining bone health have provided enough evidence to show that Indian women with polymorphicm allae at vitamin D receptor (VDR) and estrogen receptor (ER) gene loci have significantly lower bone mass. A strong association of VDR and ER gene

Polymorphism with low spinal and femoral BMD in postmenopausal Indian Women was also observed. The age related bone loss in these women was relatively at an accelerated rate as compared to that in women with non-polymorphic genotypes. The association of VDR and ER gene polymorphism with BMD was also observed in a 'closed ethnic group' (Parsee women), albeit the frequency of the polymorphic genotypes varied in this ethnic group.

Research programmes are also being pursued to study the influence of life style factors such as smoking, exercise and alcohol intake on bone health of physically active men. The initial data indicated that smoking has deleterious effect on bone mass, whereas moderate alcohol consumption was beneficial to bone mass. The exercisers demonstrated significantly lower BMI and higher BMD. The study suggests the need to educate and inform women and men of the menopausal transition and life style patterns which influence bone health. This would help them to effectively deal with problems of aging and timely seek appropriate medical care, if necessary. To facilitate this, the Institute has established an Elderly Women's Clinic to address geriatric health issues of this group of population.

## 8. Therapeutic Potential of Stem Cells

To realize the therapeutic potential of the embryonic stem (ES) cells, the Institute in 2003 embarked upon a multifaceted approach to (i) derive human ES cell lines in xeno-free environment and also by somatic cell nuclear transfer to avoid immune rejection at the time of therapy; (ii) establish ES cell lines in nonhuman primates for pre-clinical evaluation of stem cell therapy; (iii) carry out lineage specific differentiation of ES cells into germ cells, cardiac myocytes, endothelial cells etc; (iv) develop animal models for human diseases and (v) use ES cells as an in vitro model to carry out screening and study developmental toxicity of various 'lead' drugs.

Recently, success has been achieved in growing inner cell mass from human embryos on human feeders using synthetic knock out medium and knock out serum in the presence of basic FGF growth factor. These cell lines will be the first of its type grown on human feeder in xeno-free environment in India. Studies are also ongoing to identify the protein/peptide secreted by the mouse feeder layer, which keeps the cells in undifferentiated and proliferating state.

## 9. Fertility Conservation in Gonadal Insufficiency Individuals

Due to increased survival of cancer patients with more aggressive management, number of cancer survivors in reproductive age group has increased, but most of them are rendered infertile due to the cancer treatment. These individuals And other cases of gonadal insufficiency - including men who undergo repeated testicular biopsies and women who wish to delay motherhood due to changing life

style patterns will benefit if their gonadal tissue biopsy is cryopreserved for later use. To achieve genetic parenthood by assisted conception procedures. In view of this, research programmes have been recently initiated to: (i) establish protocols for gonadal tissue cryopreservation; (ii) mature germ cells in vitro by organotypic cultures; (iii) transplantation of germ cells for in vivo maturation; and (iv) create a banking facility for gonadal tissue, gametes and stem cells. Cryopreservation protocols have been developed using various cryoprotective agents. Studies on maturation of germ cells by in vitro cultures and transplantation studies are under progress.

## 10. Genetics and Reproductive Health

The research programs carried out at the Genetic Center encompass various aspects related to human health, with the primary aim of preventing birth of a genetically defective baby. Research has been undertaken in areas of recurrent spontaneous abortions, congenital heart diseases, fragile X syndrome, chromosomal aneuploidies etc. ELISA for HbA2 has been developed which will be useful to screen haemo-globinopathies in large number of individuals at a time. Karyotyping and FISH are routinely done to study structural and numerical defects including mosaicism. Telomeric FISH is being used to study cryptic chromosomal rearrangements in RSA patients. Comparative genomic hybridisation and paint-FISH will be used for carrying out genomic analysis in future. Prenatal diagnosis is offered to detect various genetic disorders and research programmes are being established to establish pre-implantation diagnosis for select syndromes.

## 11. Structural Biology

The main aim of this programme is to delineate the functional domains and bionutralization epitopes of proteins involved in various reproductive processes. Extensive studies have been done to develop the compounds that can either antagonise or mimic the action of hCG and FSH, two major glycoproteins, having potential applications as contraceptives or as fertility potentiating drugs. Conformational epitopes of hCG and FSH have been identified on these hormones. Role of disulphide bonds, present in hCG and hFSH, in the formation of heterodimer and receptor binding has also been identified.

Identification of peptides corresponding to functional domains on the hormone specific  $\alpha$ -subunit of hCG and FSH and subsequent demonstration of their ability to inhibit the binding of native hormones to their receptors has opened up the possibilities of developing new hormone antagonists. L2 loop of hCG and FSH has been identified as immuno-neutralization epitope, as antibodies raised to region corresponding to 38-58 hCG and 31-52 FSH bound and neutralized FSH activity.

Besides gonadotropins, gonadotropin receptors are also being explored for the presence of druggable targets in their molecular architecture. The regions 9-30, 216-235 and 285-309 of FSH receptor (FSHR) have been delineated as the critical regions for receptor activation. The peptide corresponding to the region 20-30 of FSHR could inhibit the binding of FSH to the receptor very effectively. Having identified the functional domains on the hormone as well as on the receptor, attempts would be to employ structure based in-silico methods to target these regions to design a low molecular weight compound having hormone modulatory activity.

## 12. Bioinformatics

With increase in the quantum of DNA and protein sequences from various species submitted to the databases on the public domain, it is now possible to identify and characterize novel genes, proteins and predict their structure and function, determine protein-protein interactions, nucleic acid-protein interactions in silico using bioinformatics tools. Recently, biomedical informatics center has been set up at the institute, which is equipped with dedicated computers and internet connectivity. The software includes packages for molecular modeling and docking, GCG sequence analysis and Sequels. This center would facilitate understanding the structure-function relationship for novel molecules identified at the Institute and elsewhere for their potential applications as contraceptives, fertility potentiating drugs, diagnostic/therapeutic tools for various pathologies and microbicides. Efforts will be made to develop a national database for proteins/nucleotide sequences of relevance in reproduction. The center would also provide training and services to scientists from other institutions, particularly from medical colleges.

## 13. Preclinical Reproductive and Genetic Toxicology

The Institute has established an “Advanced Centre for Research in Preclinical Reproductive and Genetic Toxicology” to evaluate the safety of new drugs, devices and vaccines. Facilities have been established to carry out general, reproductive and genetic toxicity including acute, sub-chronic and chronic toxicity, fertility testing, teratogenicity, prenatal and postnatal developmental toxicity, chromosomal aberration, micronucleus test and COMET assay. Few compounds including *Embelia ribes*, *Piper longum* and *Pippaliyadi vati*, which have potential for further development as antifertility agents, have been evaluated by this center. The center has also evaluated “Basant” developed by Talwar Research Foundation as an antimicrobial agent, and testosterone spray developed by CIPLA Pharmaceuticals for their potential toxicity.

The center would be a national resource for evaluating the new products for their safety, and the facilities would be offered to scientists from other institutions as well. The center would work towards creating trained manpower by organizing workshops in reproductive and genetic toxicology, and disseminate information on

related issues by organizing symposiums, seminars and through publications.

#### 14. Development of Diagnostic Tools

Development of simple, sensitive and cost effective diagnostic tests for infertility, endometriosis, premature ovarian failure, RTIs/STIs and osteoporosis has always been a high priority, considering the scope of their clinical applications in Indian context. A highly sensitive and specific pregnancy detection test, based on ELISA technology, was developed during the early eighties and this method has since been refined by increasing its sensitivity. The method has been simplified into a convenient dip and read strip test.

Urine-based methods for quantitation of estrone glucuronide, pregnanediol glucuronide, follicle stimulating hormone and luteinizing hormone have been developed as self-contained kits for infertility management. These technologies have been transferred to the industry for mass scale production and marketing.

The Institute has also developed a specific, simple, non-invasive and sensitive test for the detection of anti-ovarian autoantibodies to diagnose an autoimmune cause for idiopathic premature ovarian failure. The test has been effectively used to identify the cause of repeated pregnancy failure in women undergoing IVF-ET. Moreover, in patients with ovarian antibodies, treatment with corticosteroids resulted in complete decline in antibody titer and improved the chances of successful pregnancy, thereby proving the clinical usefulness of this test.

A test, based on sperm mitochondrial activity, acrosome intactness and/or nuclear chromatin decondensation, has also been developed to predict the fertilizing ability of human spermatozoa. Osteocalcin, N and C terminal telopeptides, pyridinoline cross links are widely used to assess bone health in the management of osteoporosis. Institute has taken initiatives to develop diagnostic tests for the bone turn over markers and has established reference norms for the Indian population.

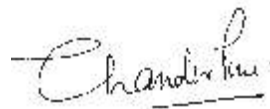
Most of the diagnostic tests for RTIs/STIs carried out in India use kits which are imported and consequently expensive. Recognizing the need for more cost effective methods, the Institute has developed an indigenous PCR-based method for the diagnosis of *Chlamydia trachomatis*, a causative agent of pelvic inflammation, female infertility and non-gonococcal urethritis in males. A PCR-based test has also been standardized for the diagnosis of human papilloma virus, one commonest factor for cervical cancer. A simple assay for elastase in urine, semen, serum or cervical specimens has been developed to diagnose inflammation due to infections. Methods to detect RTI causing pathogens such as *Candida* (both wet mount and Gram stains), *Bacterial vaginosis* (by Gram stain using Nugent's score) and *Trichomonas vaginalis* (by wet mount microscopy) have also been established. The Institute also participates in the External Quality Control Programmes to ensure accuracy of the results using indigenously developed diagnostic methods.

## 15. Dissemination of Information

The Institute realizes the importance of dissemination of information generated among various stake-holders and users such as general public, researchers, policy makers and programme managers. While the new research findings are published in indexed journals, information of common interest is disseminated through newsletters, and interactive educational meetings involving respected peers to influence the practice of community. Research findings are also shared with the community through IEC by use of posters, banners, pamphlets, booklets, charts etc during “Health Melas”, World Population Day, and HIV/AIDS Day.

The number of scientific papers published has increased over the last few years. Moreover, most of the papers have been published in indexed journals of high impact factor. This is a reflection of high quality, authenticity and relevance of the research programmes being pursued at the Institute.

The Institute also organizes national and international conferences for wider dissemination of information as well as to learn from the experiences of others. During 2006, two international conferences were hosted: International Symposium on Emerging Trends in Genomic and Proteomic Sciences; and International Conference on Actions to Strengthen Linkages between Sexual and Reproductive Health and HIV/AIDS.



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