

9. RESEARCH SUPPORT FACILITIES

State-of-the-art facilities support the Institute to pursue research in various aspects of reproductive health: (i) reproductive health clinics that provide clinical material essential for research; (ii) an experimental animal that houses various species and strains of animals; (iii) modern equipment; and (iv) a library and information center.

9.1 Reproductive Health Clinics

The Institute's clinics provide reproductive health facilities for the nearby lower middle class community. The clinic beneficiaries are a source of clinical material as well as volunteers for research studies undertaken by the Institute scientists with due approval from the Institutional Ethics Committee. The six clinics facilitate research in the areas of fertility regulation, screening and prevention of birth defects, infertility and enhancing involvement of men in the reproductive health of the family. Obstetricians/gynaecologists, pediatricians, general physicians and andrologists attend these clinics, where trained social scientists and counselors motivate and assist in providing various reproductive health services.

9.1.1 Division of Clinical Research

The Institute's clinics provide family planning services and serve as a model for integration of family planning services with other aspects of reproductive health care. Services offered routinely include awareness, screening and treatment of reproductive tract infections (RTIs), screening for cervical and breast cancer, colposcopy, pelvic ultrasonography for women and treatment of minor ailments for children under 5 years and adolescent girls.

Women attending these clinics are enrolled for clinical and basic research studies with their consent and in accordance with the ethical guidelines of ICMR.

During this year a total number of 6297 women attended the clinic for contraceptive advice and 20 children for minor pediatrics problem. Clinics provided 161 women with Cu-T 200/ Cu-T 380, OC to 239 women, condoms to 92 couples and NET-EN injection to 153 women. A total of 358 packets of OC and 8390 condoms were provided to the clinic patients and 81 pelvic sonographies were done. In the HIV OPD at B.J. Wadia Hospital, 91 discordant couples were counseled and 87 accepted condoms.

Cervical cytology Screening

During the reporting year 2966 Papanicolaou smears (cervical 1586, endocervical 1380) from 1586 women were screened for cervical cell abnormalities and infections. Of these 590 women were contraceptive non users (331 women from family planning clinics –and 206 women from endocrine infertility clinics) and 1049 women were contraceptive users (IUCD -581, Oral pills - 32, male partner condom use – 53, withdrawal method – 10, injectable contraceptive Net-En– 213, tubal sterilization – 113) women.

The cytological findings were as follows: normal /negative smears in 41.6 percent (N=660) women, inflammation in 54.9 percent (N=870) and various grades of abnormal cytology in 2.3 percent (N=19) (CIN I – 16, CIN II – 1 and Sq. cell carcinoma in 2, one with biopsy confirmation).

The overall prevalence rate of single or multiple RTIs observed by cytology was 26.3 percent (N=417) individual rates being: Trichomonas vaginalis 0.5 percent (N=8), Moniliasis 9.3 percent (N=148), Bacterial vaginosis 7 percent (N=112), Chlamydia trachomatis 9.0 percent (N=144), Herpes simplex viral infection 0.1 percent (N=2), Human Papilloma viral infection – 5.7 percent (N=91) and Actinomyces like organisms in 5.2 percent (N=30) of IUCD users.

External quality assurance in cytology laboratory for screening and reporting complied under the Indian Academy of Cytologists. One set of four slides under A and E committee was screened and 100 percent correctly correlated as per the committee report.

9.1.2 Infertility and Reproductive Endocrinology Clinic

Reproductive Disorders

Research support clinical services are being offered to infertile couples and to those with reproductive endocrine disorders. The services provided include semen analysis, PAP smears, hormone assays and ultrasonography. These couples provide biological material for clinical and basic studies of the Institute after consent and in accordance with the ethical guidelines of ICMR.

Female infertility

Services in the form of detailed history, and clinical examination, laboratory tests such as FSH, LH, Testosterone when indicated were offered to subjects who participated as controls/ or as participants for projects. PAP smears from the endocervix are done for all new cases; sperm survival tests were carried when indicated. Clinic consultations under supervision for routine service /project related visits constituted ~5580 patient visits in a year. Ultrasonographic

scans for follicular monitoring, early pregnancy detection and obstetric scans (approx 4095) were carried out during the year.

During the reporting year, the clinic enrolled 256 new cases. These included 250 infertile couples and 6 unmarried girls with endocrine problems such as hirsutism and amenorrhoea. Of these 40 discontinued treatment midway and 20 are being investigated. All these women underwent cervical smear examination for detection of cervical intraepithelial neoplasia/inflammation/STI infection. Pelvic ultrasonography (n = 4454) was done for documentation of ovulation, monitoring response to ovulation inducing agents, detection of early pregnancies, assessment of foetal well being in utero as well as for uterine ovarian morphology in women with various endocrine disorders.

In addition, post coital tests, artificial insemination homologous, endometrial biopsies and hydrotubation were carried out as part of management. Estimation of serum gonadotrophins, androgens by RIA were done to diagnose specific endocrine disorders and to select cases for various studies of the department.

One hundred and twenty-three infertile males were evaluated and managed in Male Infertility Clinic attended by two Andrologists.

Ninety new cases of infertile couples seeking treatment for infertility were enrolled.

To assess male factor status

Routine semenology for all male subjects who enrolled in the clinic was carried. Andrological examinations for 180 infertile cases 46 out of which were follow up cases were carried out by the visiting andrologists to the Institute to determine clinical diagnosis and further management. Doppler test for varicocele as done wherever indicated.

9.1.3 Elderly Women's Clinic

The service cum research community based clinic caters to services in the area of menopause and osteoporosis. Routine health services include clinical examination, breast examination, cytology screening, ultrasonography and colposcopy as deemed necessary. In addition to these services, IEC focusing on dietary intake, lifestyle modification and prophylactic bone health measures to maintain bone health is imparted to women attending the clinic. Bone density measurement facility is offered to women as part of the project activity. Approximately, 400 women have availed of these clinic facilities.

Individual and group counseling regarding menopause and osteoporosis are being held regularly at the clinic. Health talks are also being conducted in the community and through women's organizations, to increase awareness among health care providers and women in general, about menopause and therapeutic measures that can be taken so as to improve the quality of life of the elderly woman.

9.1.4 Adolescent Friendly Centers

Two adolescent friendly centers were set up in two schools as part of the project activity and these function for two days of the week for 3 hours or as required. A medical doctor and two social staff run these centers. The timings were fixed according to the convenience of the students and the school authorities. IEC material on various aspects of sexual and reproductive health including nutrition is made available at these centers and in the library of these institutions. A letterbox to answer queries of these students has also been made available. Collaborations were established for referral services.

Common questions asked through the letter box

Issues	No. of questions asked
•Menstruation	59
•HIV/AIDS	21
•Masturbation/wet dreams	13
•Sexual Intercourse	38
•White discharge	5
•Rape	6
•Acne	10
•Attraction to opposite sex	16
•Homosexuality	1
•Others	45

A number of adolescents have visited these centers for various medical and psychological problems. Teachers also refer students and many parents have visited these centers for counseling along with their wards. Thus indicating a big demand for information on sexual and reproductive health matters.

The table below shows the type of complaints students presented during the current one year.

Services	Number of clients
Information	48
Menstrual complaints	28
Problems related to growing up	19
Behavioural problems	20
Wet dreams/ Sexual intercourse	3
Skin Infections	14
White discharge/ pruritis	8
Premarital counseling	1
Pregnancy and medical abortion	1
General Health	18
Homosexuality	1
Total	150

9.1.5 Genetic Counseling Services

The Genetic Research Centre has been a leader in assuring access to quality genetic services in western Maharashtra. Genetic disorders affect individuals from birth to old age and the principles cut through every medical subspecialty; therefore it is a challenge to health care providers. It has played a crucial role in educating patients, their families and the medical profession. It has caused awareness in public and helped in decision making. Anticipatory guidance, especially for the Fragile X syndrome has also been provided.

As part of the ongoing activities, genetic services provide secondary prevention i.e. giving preconceptional folic acid in prevention of ventral and dorsal defects and reproductive loss and at the tertiary level, where counseling provides information on carrier detection and prenatal diagnosis.

Over the last one year, a total of 290 cases were registered at the genetic clinic. These included 32 cases of mental retardation, 75 cases for prenatal diagnosis, 78 individuals having two or more abortions, 40 cases of short stature and primary amenorrhea, 51 cases of male infertility, 11 case of ambiguous genitalia and 3 cases of congenital heart disease. Further, 400 children from special schools of Bombay Municipal Corporation were examined and evaluated for Fragile X syndrome. Follow up visits were 505. Cytogenetic analysis was carried out in 261 cases.

Since genetic disorders are rarely curable, the best approach for management is by prevention, counseling and prenatal diagnosis either by 3 D ultrasound or by molecular markers eg. Microdeletion of Ch22.

The clinical geneticists' role in identifying rare syndromes in index patients or through old records is crucial for accurate genetic counseling and prenatal diagnosis and management of genetic disorders. Some interesting cases are highlighted below.

i) Correlation of diagnosis with molecular markers for Genetic Counseling – Lipodystrophy:

Generalized Lipodystrophy of the Berardinelli-Seip type (BSCL) is a rare autosomal recessive human disorder with severe adverse metabolic consequences. Two genes one on chromosome 9 and other chromosome 11 has recently being identified. We report our case of Lipodystrophy who had hepatic dysfunction, hyperlipidemia diabetes mellitus with loss of subcutaneous fat, phlebomegally and hirsutism. (Fig. 151 & Fig. 152)

Molecular analysis performed in this family for BSCL 1 & 2 group revealed mutation in BSCL 1. She had no heart disease or mental retardation. This analysis was carried out by Prof. Lionel Van Maldergem. This aided in appropriate genetic counseling.



At 9 Months

Fig.151



At 26 Years

Fig. 152

ii) *Johanson Blizzard Syndrome – Usefulness of 3D USG:*

Johanson Blizzard Syndrome is a rare autosomal recessive disorder characterized by aplasia of alae nasi, bilateral sensory neural deafness preauricular tags ear pits and speech defects. A 29 year old gravida 1 para 2 was referred in her second pregnancy. Her first child had all the features of Johanson Blizzard Syndrome. The mother was seen at 18 weeks of pregnancy. A 3D USG using voluson 73 DD machine revealed a prominent normal nose and normal ears, thus ruling out above syndrome courtesy Dr. C. Lulla (Fig. 153 & Fig. 154). She delivered at term a normal male child of 3 Kg. weight. We believed that 3D USG for face can be used as a diagnostic marker for this syndrome.

3D USG showing normal face & ears



Fig. 153



Fig. 154

iii) Importance of documentation of old records for prenatal diagnosis - Escobar Syndrome – Popliteal pterygium Syndrome:

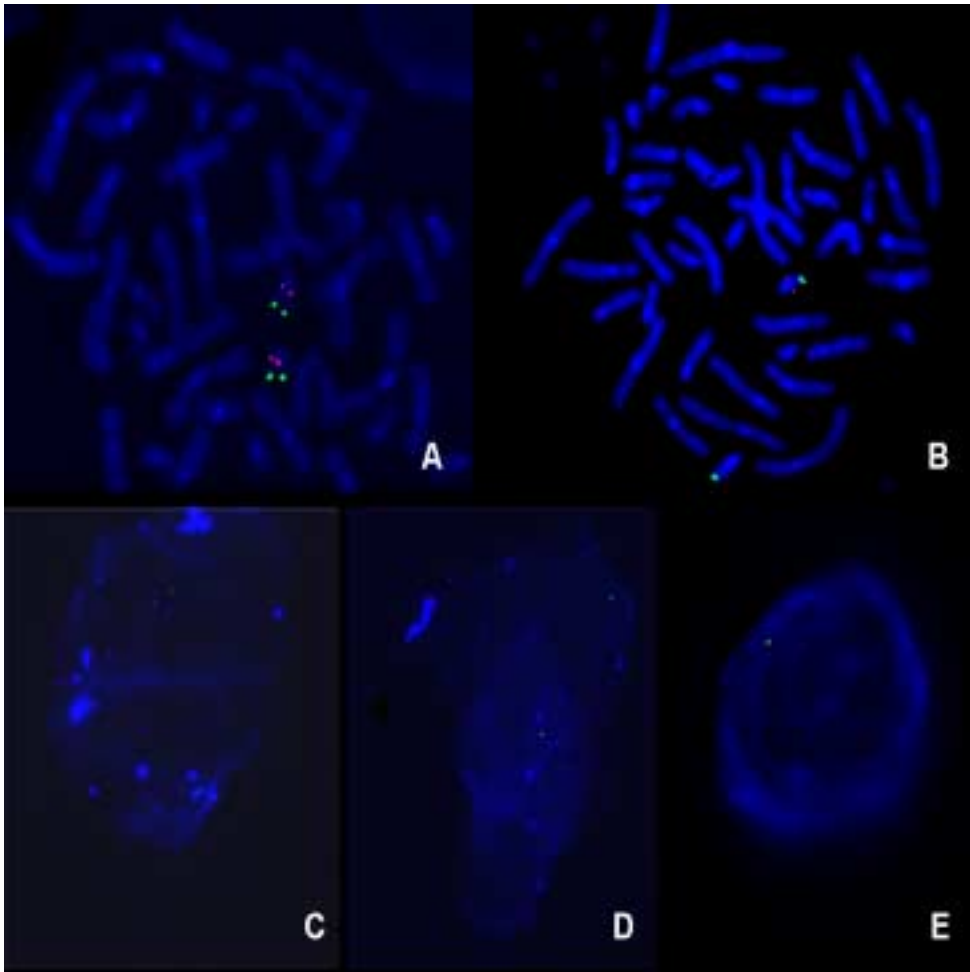
This well defined syndrome is complex and consists of popliteal pterygium, digital anomalies valgus deformity in feet, cleft lip and cleft palate. The baby with the above anomaly was born to the non-consanguineous parents and had expired. When seen by us, the mother was pregnant. On going through old records, a diagnosis of Escobar syndrome was confirmed on x-ray findings. Prenatal diagnosis was offered to the couple by USG at 16 weeks. The USG revealed normal fetus.

This case stresses the importance of preserving old records for accurate diagnosis and counseling and prenatal testing.

iv) Usefulness of advanced cytomolecular techniques like FISH and 3D USG in prenatal diagnosis:

Microdeletion of chromosome 22 is responsible for DiGeorge syndrome, velo cardio facial and conotruncal defects. We report our experience in prenatal diagnosis in a family who had lost two children with complex cyanotic heart disease. FISH analysis in the couple revealed that mother was mosaic for the microdeletion of chromosome 22q11 in 10% of cells. Prenatal diagnosis was offered to her in her third pregnancy. On routine Ultrasonography at 16 weeks the four chambered view of the heart was normal. However before any further tests could be performed she aborted. FISH studies on the heart tissue revealed 22q11 microdeletion with three different cell lines (Fig. 155).

This suggests the importance of performing FISH studies when there is a history of congenital heart disease, even though Ultrasonography showed a normal four-chambered view of the heart.



**Fig. 155 A. FISH Studies Showing normal Chromosome 22
 B. Metaphase showing microdeletion of Ch22q11.2
 C. Heart tissue showing normal Ch22
 D. Heart tissue showing microdeletion Ch22q11.2
 E. Heart tissue showing monosomy 22**

9.1.6 Institutional Clinical Ethics Committee

Clinical studies involving humans are reviewed by the Institutional Ethics Committee as obtaining approval from the Committee is an essential prerequisite to initiation of all studies. The Committee is multidisciplinary and multisectorial in composition with 11 members from outside the Institute. All members are fully cognisant with the prevailing local, social, legal and cultural norms. Each project is evaluated from the point of possible risk to subjects, proper justification, expected benefits, adequacy of documentation, procedures of informed consent, including appropriate translations into local languages, ensuring privacy, confidentiality and justice. The Committee also scrutinizes the competence of the investigators undertaking research work and reviews the

progress of the approved study proposals and modifications, if any, introduced during the course of the studies, adverse events and results of research work.

Thirty six projects were reviewed and ongoing as well as modified projects scrutinized during the meetings held through the reporting period.

9.2 Electron Microscopy Department

The Electron Microscopy Department is a service-oriented unit of the Institute. One hundred and eight reproductive tissues of human, monkey, rat & mice belonging to various ongoing projects at the Institute were embedded and semi thin sections were prepared. Selected areas were used to cut ultra thin sections. Representative areas were saved using CCD camera and analyzed using analysis software, on TECHNAI 12. The department also undertook ultrastructural evaluation of dental tissue for a MD thesis (Nair Hospital) on request.

Besides conventional electron microscopy, immunogold labeling of antigens of interest at the ultrastructural level was standardized using 10nm gold particles. Ultra thin sections of epoxy embedded & osmicated tissues were found suitable for simultaneous fine structure preservation and immunogold labeling.

This procedure was used to localize estrogen and progesterone receptors in rat testis (ongoing study with Primate Biology Dept.) and 27 kDa epididymal protein in rat caudal sperm (ongoing study with Gamete Immunobiology Dept). Studies are ongoing to localize oviductal glycoproteins in human fallopian tube (ongoing study with Biochemistry Dept) and 57 kDa-sperm protein (ongoing study with Immunology Dept) by the same method.

9.3 Department of Flow cytometry

The flow cytometer facility was made available to researchers both from within and outside the institute for their research projects. DNA and cell cycle analysis, apoptosis and immunophenotyping, using primary cell suspensions as well as cell lines for analysis.

During the current year, various investigators availed the facility and approximately 1500 samples were analysed for institutional research projects, for their projects, 84 samples from the Institute of Immunohaematology (ICMR) and 50 samples from BARC were also analyzed. An ongoing collaborative project with the Pharmacology Department of BYL Nair Hospital, Mumbai, to study the apoptotic changes in cell lines has also been undertaken and more than 300 samples have been analyzed so far.

9.4 Experimental Animal Facility

The Institute has a well maintained animal facility distributed over three floors and different species of laboratory animals such as mice (785), rats (533), rabbits (127), bonnet monkeys (78) and marmosets (133) are being maintained and taken care of by qualified and trained staff including weekends and holidays.

Table 17: Animals bred and supplied during the year (April 2003 – March 2004)

Sr. No.	Species	Animals bred	Animals supplied	Total strength as on March 2003
1	Swiss Mice	1191	924	589
2	Balb/c Mice	566	162	196
3	Holtzman Rats	1362	941	533
4	Rabbits	55	56	127
5	Bonnet Monkeys	-	-	78
6	Marmosets	33	38	133
7	Sheep	-	1	1

9.4.1 Institutional Animal Ethics Committee

The Institute is registered for breeding and conducting experiments on animals (Control and Supervision) with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (vide Registration No. 78/1999/CPCSEA dated 11 March 1999).

The Institutional Animal Ethics Committee, including CPCSEA nominee, reviews the research activities, which involve the use of laboratory animals. The Committee also ensures that laboratory animals are housed in an appropriate environment, properly taken care of and used only when other *in-vitro* methods cannot provide the desired information. In brief, the Committee ensures that the concept of 3R's – Reduction, Refinement and Replacement in the use of animals in research is followed.

During the year animals were supplied to 24 different projects approved by the Institutional Animal Ethics Committee.

9.4.2 National Center for Primate Breeding and Research

The establishment of a “National Center for Primate Breeding and Research”, at Sasunavghar, Dist. Thane has been approved by the Government of India. The creation of the center is not only essential but also inevitable due to current restrictions on trapping of monkeys from the wild life and the need for nonhuman primates of defined health status and pedigree for research work. The Institute has acquired 25 acres of land at village Sasunavghar, which is quite suitable for the purpose, being away from human habitation and covered with green forest. An Expert committee, constituted by the ICMR, had recommended to accord high priority to develop the Center. This center would meet the current and anticipated future requirement of the Institute as well as that of the national scientific community to conduct research on interdisciplinary biomedical problems significant to human health and where nonhuman primates are the animal model of choice.

9.5 Library and Information Center

The Dr. G. M. Phadke Memorial Library and Information Center houses an exclusive collection of books, journals, databases, reports etc. on subjects encompassing all aspects of reproductive health care. A collection of manuals and books on basic techniques in molecular biology, immunology, cell biology, stem cell research, bioinformatics etc. complement the subject specialities. The library is open for extended hours on all working days to information seekers. Reference, interlibrary loan, on-line and database (Medline and Popline) services are offered without any charge. Reprographic services are also available. The Institute is endeavoring to provide fully automated library, referral, and web-based services to scientists. Information on books and journals procured and services offered during the year is given in Table 14.

Table 18: Books and journals procured and services extended during the year

Acquisition:	
Books	27
Journals subscribed	90
Services:	
No. of users from other Institutions	1560
No. of photocopies provided to readers	1,15,864
No. of pages copy printing	1,71,930

9.6 Biostatistics and Computer Section

The department takes active part in planning, conducting and analysis of clinical trials, necessary laboratory investigations and coordinates the activities of various departments of the Institute. Statistical staff are involved in the preparation of random allocation lists, designing of analysis, abstraction of salient features from protocols for the benefit of various departments, organization of reminder systems to ensure adherence to plan procedures, maintenance and initiation of corrective measures, as and when required.

Computers are being increasingly used in the department for database management and analysis, desktop editing and printing (DTP) of research papers, annual reports, technical reports, slides, posters, project proposals etc. Department is training the users and also monitors the process. The department has facility of Computer Room equipped with three Pentium III computers, one laser printer, one DeskJet colour printer, one scanner and one photocopier.