

## REPRODUCTIVE HEALTH

Realizing the significance of good reproductive health in ensuring overall well being of an individual and the society, the Government of India, jointly with 149 other countries of the United Nations Organization, has decided to achieve the Millennium Development Goals (MDGs) by the year 2015. These goals are set to achieve: (i) eradication of extreme poverty and hunger; (ii) primary education for all; (iii) gender equality and women empowerment; (iv) reduced child mortality; (v) improved maternal health; (vi) reduced incidences of HIV/AIDS, malaria and other diseases; (vii) environmental sustainability; and (viii) a global partnership for development. Of these eight goals, three directly address to the issues related to reproductive health, reflecting the urgency on the part of governments to reduce the burden of reproductive ill health. Countries worldwide are striving hard towards reduction of infant and child mortality by two-thirds (goal 4); reduction of maternal mortality by three-fourth (goal 5); and to reverse the spread of HIV prevalence in 15-24 yr olds by 25% and infants infected with HIV by 50% (goal 6).

To ensure that the MDGs are achieved, the Council's National Institute for Research in Reproductive Health (NIRRH) at Mumbai has initiated several research programmes with special focus on reducing the burden of reproductive ill-health due to unmet need of contraception, unwanted and unsafe pregnancy termination, infertility and other reproductive disorders, increasing incidence of reproductive tract infections (RTI) including sexually transmitted infections (STI) and HIV/AIDS, and geriatric health problems like osteoporosis in men/women. The research programmes are in accordance to the needs and perspectives of people taking into account the changing patterns of adolescent sexuality and fertility, increasing incidence of sexually transmitted infections including HIV/AIDS and concentration of reproductive ill-health among urban slum, rural and tribal areas. Research in the areas with a promise to improve the reproductive health has been initiated using newer

technologies such as bioinformatics, system biology, structural genomics, proteomics and genomics.

The Council is also undertaking studies in the field of maternal and child health. Home based management of young infants at village level by trained health workers is being attempted in five states of the country. The Council has developed National Guidelines for Assisted Reproductive Technology Clinics in the country. National guidelines have also been developed for management of reproductive tract infections.

### FERTILITY REGULATION

#### BASIC RESEARCH

#### Identification and Characterization of Sperm Antigens

##### (i) Using neonatal tolerization

Neonatal tolerization was used to raise antibody probes, which were used to delineate domain specific epididymal sperm proteins. Animals were tolerized at days 0 and 5 with rat testicular proteins and further immunized with either intact or soluble fractions of head (HI/HS) and flagellar domains (FI/FS) of rat caudal sperm. Western blot analysis using sera of tolerized immunized (TI) animals showed epididymis specific reactivity. The sera of animals in different groups identified proteins in the range of 27-200 kDa.

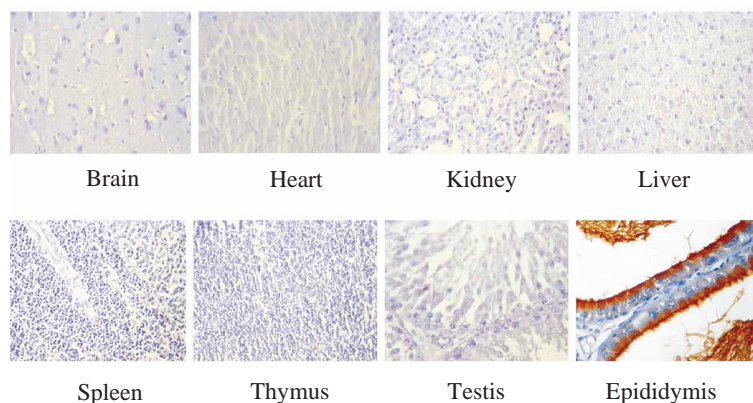


Fig. 1. Tissue specificity of sperm proteins

The sera were of the agglutinating type and the pattern of agglutination was either radial or comet shaped. A representative data of the immunohistochemical reactivity of TI sera with rat brain, heart, liver, kidney, spleen and thymus is shown in Fig. 1. No tissue apart from cauda epididymis showed any positive staining suggesting that the antigens recognized by all the sera were of epididymal origin.

### (ii) Using antisperm antibodies

Using vasectomy induced autoimmunity approach, a sperm auto antigen, termed TSA70 was identified and immunochemically characterized. It was found to be a testicular auto antigen of ~70 kDa expressed post meiotically in stage specific pattern and is evolutionarily conserved across the species. The sequence of TSA70 showed homology with cenexin molecule. This is the first report showing the presence of cenexin on sperm.

### (iii) Using proteomics approach

Proteomics was employed to identify differentially expressed sperm proteins acquired during epididymal maturation. The proteome profiles of rat testicular sperm were compared with caudal epididymal sperm as well as with proteins from different domains (head and flagellum) of caudal epididymal sperm. The profiles obtained were analyzed as many as 404 protein spots were resolved from testicular sperm and 495 from cauda epididymal sperm. Several spots were specific to epididymal sperm. Of these spots, two (AS1 and AS2) are being sequenced.

## Role of a Novel Androgen Regulated HoxB2 Containing Gene Expressed in the Epididymis

A sperm specific protein designated HOXBESS was, identified by screening an epididymal cDNA library, using an agglutinating antibody raised against washed human sperm. The sequence of the positive clone showed homology with the conserved region of transcription factor Hox-B2. The epididymal specific expression of the 30kDa protein, (presence of a single 2.5kb transcript), its androgen dependency, conservation across the species and presence on the sperm surface were established. Acrosome reacted sperm of various species indicate a probable role for the protein in sperm-egg interaction and sperm maturation. The data indicated regionalized and cell

specific expression of HOXBESS that shares epitope identity with Hox-B2 transcription factor from embryo. Specific cytoplasmic localization, the difference in the size of the cognate epididymal protein from the HOX-B2 transcription factor expressed in the embryo and presence on the sperm suggests the role of HOXBESS as an effector molecule rather than a transcription factor.

Using the 5'RACE approach, the full length sequence of 1.657 kb was obtained from rat epididymis. The full length mRNA as well as its putative protein sequences was submitted to the NCBI GenBank and ascribed the accession nos. DQ399532 and ABD73307 respectively. Sequence analysis revealed homology to HOX-B2 transcription factor with an upstream extension of 560bp. Sequence analysis using bioinformatics tools showed a start site at 218bp and a stop site at 1135bp. Kozak sequence and poly<sup>+</sup>A tail were also identified. The deduced putative protein (31.5 kDa) with a precipitation index (pI) of 10.5 from an ORF of 917bp. A mitochondrial targeting peptide (1-30 A.A.) correlated with the immunolocalization of the protein on the midpiece of rat, monkey and human spermatozoa. A nuclear localization signal and the immunodominant region of 20 amino acids could also be identified. Potential transmembrane segments in the 305 aa sequence correlated with the integral membrane bound extra-cellular (secreted) form of the predicted protein location.

The protein exhibited differences in the amino and carboxyl termini from the conventional transcription factor, probably explaining the differences in their function. Sequence analysis of full length of the Hox-B2 containing sperm protein confirmed the earlier findings based on *in vivo* and *in vitro* observations. The observations further confirm that HOXBESS has a role to play in sperm function during fertilization.

## Studies with 80kDa Human Sperm Antigen and Its Synthetic Peptides

The 80kDa human sperm antigen (HSA) has been reported to be a sperm specific protein responsible for inducing immunological infertility. Active immunization with purified antigen induced reversible infertility in male and female rats.

The immunohistochemical and immunofluorescent studies have suggested that 80kDa HSA is sperm specific antigen and its expression is developmentally regulated

by testosterone. Synthetic peptides of this antigen immunobiologically mimic the native protein and immunogenic and active/passive immunization impaired fertility in rat, rabbit and marmoset. Thus, the use of 80kDa RSA and its synthetic peptides is suggested for development of an antifertility vaccine.

### **Modulation of c-kit Proto-Oncogene Function during Spermatogenesis in Mice**

Infertility affects 5-7% of men, of these 30% are due to genetic factors, 10% due to 'Y' chromosome microdeletions and the remaining 90% are due to defects in autosomal genes. C-kit is one such autosomal gene. In certain cases, the infertility could be attributed to the functional defects of C-kit expression, since C-kit mutant mice are reported to be infertile. Study was conducted under an Indo-US programme to evaluate the role of C-kit during different stages of spermatogenesis at the molecular level using mice as an experimental animal.

During the year isolation of pure population of spermatogonial cells (SGCs) was done and they were used to study the role of c-kit in germ cell survival using serum-free short-term culture system. The results indicated that c-kit and its ligand, the stem cell factor (SCF) facilitate the survival of SGCs. Study of expression of c-kit during different stages of development revealed that it increased till day 60 following which no significant change was found suggesting maturation-associated expression. Method for the *in vivo* transplantation of SGCs into the seminiferous tubules *via* rete testis of busulfan-treated infertile mice has also been standardized. Sequence specific gene silencing in mice testis induced by electroporation-mediated transfer of short interference RNA (SiRNA) is being studied to evaluate the role of c-kit in SGC survival and differentiation.

### **Global mRNA Expression Studies on Apoptosis Related Genes in Human Endometrium during Menstrual Cycle**

Deregulation of apoptosis in endometrium can result in aberrant proliferation and related diseases such as hyperplasia. Identification of key genes involved in endometrial apoptosis may help in elucidating prognostic markers for hyperplasia. Immunohistochemical localization of apoptosis regulating genes of the Bcl-2 family (such as Bcl-2 and Bax) in human endometrium

during various phases of the menstrual cycle was undertaken earlier at NIRRH, Mumbai. To further elucidate the molecular pathway of apoptosis in endometrium, global expression of apoptosis related genes was investigated during the year by cDNA macroarray. Endometrial biopsies were collected from women volunteers at follicular (F), mid luteal (ML) and late luteal (LL) phases. Total RNA was extracted from the endometrial samples, cDNA was synthesized using specific primers and labeled with <sup>32</sup>P. Following hybridization with human apoptosis cDNA array on nylon membrane, the spots were visualized by phosphor imager. The array contained 205 genes involved in various apoptosis- regulating pathways. Increase in the intensity by 2-fold or more was considered to be significant. Exclusively 30 genes were upregulated in ML phases and 15 genes were upregulated in LL phases while 38 genes showed 2-fold rise in both ML and LL phases as compared to F phase. Preliminary analysis of the data revealed prominent role of the cell cycle regulating protein kinases and intracellular kinase network members in endometrial apoptosis. Further investigations are underway for validation of the results.

### **Dynamics of Early Folliculogenesis: Differential Gene Expression in Mouse Ovaries**

The ovary contains a pool of primordial follicles containing oocytes arrested in meiosis and which are the source of developing follicles for the female. Transition of arrested primordial follicles to primary and secondary follicles is a crucial process in female fertility. Relatively few genes which control these events are known, thus identification of additional genes expressed during follicular development may reveal key players in this process. To investigate this RNA was extracted from ovaries of neonatal mice on days 2 and 4 and the gene expression profile was analysed by cDNA. The results revealed that 30% of genes were differentially expressed during transition of primordial to primary follicles. Amongst these genes, upregulation of cell cycle regulators, neurotransmitters and signal transduction elements was predominant. Genes responsible for growth, cell-cell communication and proliferation were also found to be upregulated. Further, to substantiate the gene expression results, the immunolocalisation of proteins such as GDF-9,

(a differentiation marker), PCNA, (a marker for proliferation) and anti Mullerian hormone, (a growth regulator) were studied in the ovaries of neonatal mice. The results demonstrated that multiple gene cascades were involved during early folliculogenesis.

### Mechanism of Action of Follicle Stimulating Hormone Binding Inhibitor (FSHBI) and its Synthetic Analogue Octapeptide (OP)

Gonadotropins FSH and LH play an important role in the proliferation and development of the follicles. Several autocrine/paracrine factors secreted by the ovary, directly or indirectly regulate the gonadotropin action. FSH binding inhibitor (FSHBI) purified and studied at NIRRH, is one such factor, which modulates FSH action by binding to its receptor. It is a ~ 4kDa protein and its N-terminal 8 amino acid sequence is an octapeptide (OP). Native FSHBI purified from human ovarian follicular fluid was shown to induce atresia in developing follicles. OP has also demonstrated a biological activity similar to native FSHBI. Study was conducted to elucidate the mechanism of action of FSHBI/OP in swiss mice. For this two pathways of apoptosis namely, the membrane receptor pathway and mitochondrial pathway were studied using flow cytometry and immunohistochemistry. Results indicated an overall increase in apoptotic population in atretic and FSHBI/OP treated groups as compared to the normal group. Interestingly, in the FSHBI/OP treated groups the membrane receptor pathway was dominant while in the atretic group it was mitochondrial pathway.

## CLINICAL RESEARCH

### Acceptability and Continuation Rates of 2 Monthly Injectable Contraceptive: Norethisterone Enanthate

At the initiative of Department of Family Welfare, the NIRRH, Mumbai is continuing a multicentric study on 2 monthly injectable contraceptive–norethisterone enanthate (Net-En) among women across the country.

Observations of the study based on 15,228 women months of use revealed that continuation rates at 12 and 18 months were 65.2 and 54% per 100 users respectively (Fig. 2). About 89% women said that the

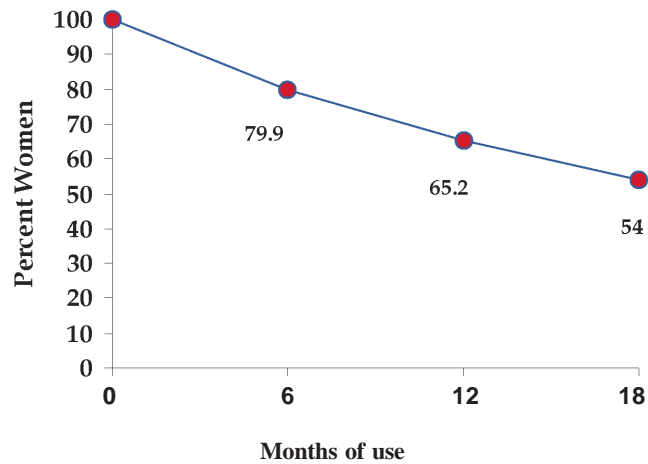


Fig. 2. Cumulative continuation rate of Net-En injection

injectable contraceptive should be made available in the National Family Welfare Programme as it is convenient to take bimonthly injection instead of contraceptive pills. Over 79% of them recommended this method to their friends and relatives and 80.5% women were satisfied with the use of this method. During the reporting period, 409 women have completed 2 yr of injection use. The method was found to be very effective since there have been only three pregnancies reported so far. The major reason for discontinuation was migration and loss to follow up because of floating population.

Protocol amendment for bone mass density (BMD) and evaluation by DEXA among Net-En users was made. Some of the women's health groups raised issues about decrease in BMD among women who used 3 monthly injectable contraceptive, depo-provera. In view of this, NIRRH has initiated BMD evaluation at 7 centres.

### Clinical Trial with Intravasal Injectable Male Contraceptive

The RISUG (reversible inhibition of sperm under guidance), an intravasal injectable male contraceptive has been developed by the Council under GLP and GMP conditions and the same has been tested for its genotoxicity and mutagenicity. The data indicated that RISUG is safe in terms of genotoxicity, mutagenicity and carcinogenicity and is safe for clinical use. The Council plans to conduct extended Phase-III clinical trials with RISUG.

## OPERATIONAL RESEARCH

### Interventions in Urban Slums for Enhancing Participation of Men in Reproductive Health

Realizing the need for involving men in the family welfare program, the draft National Population Policy (2000) suggested a holistic approach and stressed on male involvement particularly in the context of prevailing gender inequality.

Operational research is being undertaken by NIRRH, Mumbai on the couples in urban slums to (1) identify program strategies (addressing only the men or couples together) for effective participation of men in programs aimed at improving reproductive health (safe motherhood, family planning/contraceptive use, RTI/STI and HIV/AIDS) of couples and (2) plan and evaluate interventions for enabling couples to gain correct knowledge about reproductive health issues concerning men and take appropriate actions to seek and avail reproductive health services.

So far, information has been collected from 1755 couples (both husbands and wives) from three health post areas (Mohili village, Bail Bazar and Asalfa village). About three-fifth of men were 19 to 39 yr old while 41% women were aged 17 to 29 yr. About 39% men were unskilled, while most (91.4%) of the women were housewives. Two-fifth of couples had three or more living children. Nearly one-fifth of husbands and one fourth of wives mentioned that they prefer to have more sons than daughters indicating gender bias. About one-fifth of wives reported that they had past history of abortion of which 12% had induced abortions. A little more than half (54%) of husbands and 47.8% wives reported that husbands accompanied wives for their antenatal check-ups.

A little more than two-fifths of husbands (43.4%) reported that their wives had undergone tubectomy and

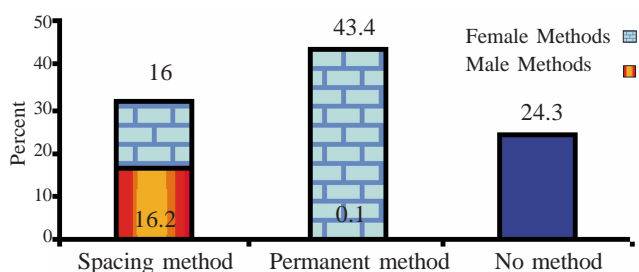


Fig. 3. Current contraceptive use

10.1% husbands reported that they are using condom (Fig. 3). Only one person had undergone vasectomy. Regarding future intentions to use contraceptives, 30% husbands and 26.7% wives reported that they would not like to use contraceptive measures.

Regarding knowledge of reproductive health issues, 97.4% husbands and 96.4% wives were aware of HIV/AIDS, 74 % husbands and 40% wives were aware of STDs and 43% husbands and 49% wives were aware of infertility.

The findings suggest that in order to enhance male responsibility in reproductive and sexual health matters (including safe motherhood, better contraceptive usage and health seeking behavior), the intervention strategies should address issues relating to gender equality and spousal communication.

## INFERTILITY AND REPRODUCTIVE DISORDERS

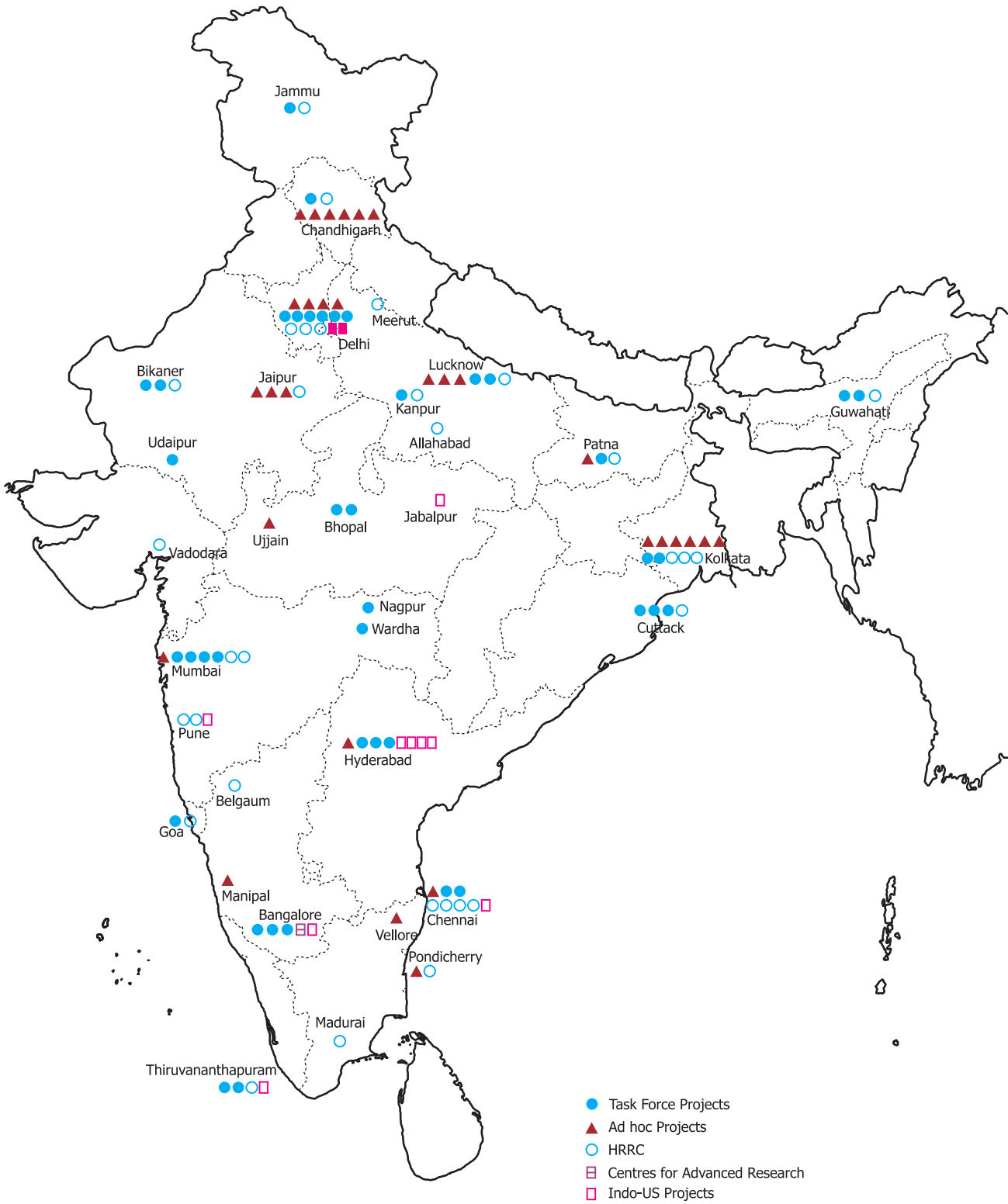
### FEMALE INFERTILITY

#### Studies on Polycystic Ovary Syndrome

#### Studies on Genetic Aspects of Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is an important endocrine disorder leading to infertility. A genetic susceptibility to the condition has now been well recognized. Evidence indicates that a small number of key genes may be contributing to predisposition to the syndrome, which in conjunction with environmental factors, may lead to its varied phenotypes. Study was carried out with the specific objective of determining the susceptibility loci for PCOS with particular emphasis on two of its important associated features *viz.*, hyperandrogenicity and obesity. Overall objective was to elucidate the associated genetic mutations and polymorphisms in Indian context in particular.

Probands for the study included women in reproductive age with oligoamnenorrhea/chronic anovulation and hirsutism with or without obesity. Polycystic ovaries were confirmed through ultrasonography. A normal control group in



MAJOR ICMR RESEARCH PROJECTS IN REPRODUCTIVE HEALTH

reproductive age and a grossly obese (BMI>35) control group were also included for comparison. The following candidate genes were analysed (i) CYP11A1 which encodes cytochrome P450 side chain cleavage enzyme, (ii) CYP17 which encodes the 17  $\alpha$ -hydroxylase enzyme and (iii) leptin, the obesity (OB) gene.

Coding sequences of CYP11A1 did not reveal any variants with regard to PCOS. Screening of the promoter region showed marked variations in frequency of the pentanucleotide (tttta)<sub>n</sub> repeat polymorphism in Indian women compared to that reported in the West. The 6 repeat allele which has been reported to be associated with raised androgen levels, was found to be predominant in Indian women, as against the 4 repeat allele which is predominant in Western population. Analysis of CYP17 showed significant association of PCOS with a T>C polymorphism in the promoter region. Leptin showed no variants in its coding region. In gross obesity, however, a novel variant was identified in exon 2 (accession number DQ054472). Screening of the non-coding exon 1 of the gene was also carried out in PCOS and control cases. A high prevalence of A>G polymorphism was found. Fig. 4 shows the heterozygous A>G polymorphism seen in a PCOS case. The study is being continued to determine the

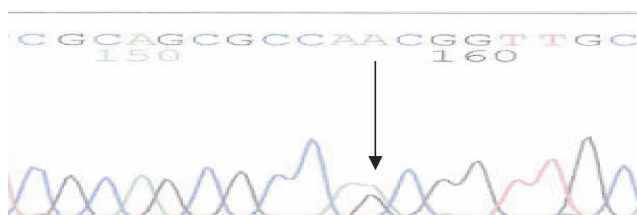


Fig. 4. Heterozygous A>G polymorphism in leptin gene seen in a case of PCOS

association of polymorphism with PCOS and associated obesity.

### Genes in Women with Premature Ovarian Failure

Study was undertaken with the aim to determine association of mutations/premutations in the FMR1, inhibin and FOXL2 genes in women with idiopathic premature ovarian failure (POF) and to identify the best candidate gene for determining predisposition to this condition. Seventy women aged <40 yr, with secondary amenorrhoea, elevated serum FSH levels (>40 mIU/ml

and normal karyotype and 30 regularly cycling fertile women were enrolled along with 25 family members of 19 index cases. Nine women with poor ovarian reserve (POR) were also recruited.

Screening of inhibin alpha gene by RFLP and SSCP analyses did not reveal any mutations. Inhibin beta A gene also did not reveal any mutations. Inhibin B levels were found to be significantly decreased in the POF patients as compared to controls. However, no significant differences were observed with respect to the POR group.

Having detected the C>T transition in the coding region of the FOXL2 gene, polyalanine tract of this gene was screened for mutations as expansion, deletion and single base pair substitutions however, no mutations were detected.

Sizing of CGG repeats in the FMR1 gene was done by PCR and PAGE. All patients and controls have been found to have CGG repeats in the normal range of 5-50. It was observed that 35-40 CGG repeats were most prevalent in Indian population. Study is being continued for screening of newer genes associated with ovarian function.

### Assessment of Gene Mutations Associated with Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is an endocrine dysfunction characterized by deficiency of enzymes crucial for synthesis of steroid hormones by adrenals. More than 95% of the cases are known to be caused due to deficiency of the enzyme 21-hydroxylase. CYP21, the gene encoding 21-hydroxylase enzyme is located in close proximity to a pseudogene called CYP21P with approximately 98% homology. CYP21P is an inactive pseudogene whereas CYP21 encodes the active 21-hydroxylase enzyme. The CYP 21 gene shows a high degree of variability in copy number as well as substantial polymorphism at the DNA level. A total of 19 mutations and 29 polymorphisms have been identified in this gene in different populations. Data on Indian population is, however, sparse. Study has been undertaken with the specific objective to identify all the mutations and polymorphisms in the CYP21 gene in Indian cases with classical and non-classical CAH. A method based on PCR-sequencing is being set up

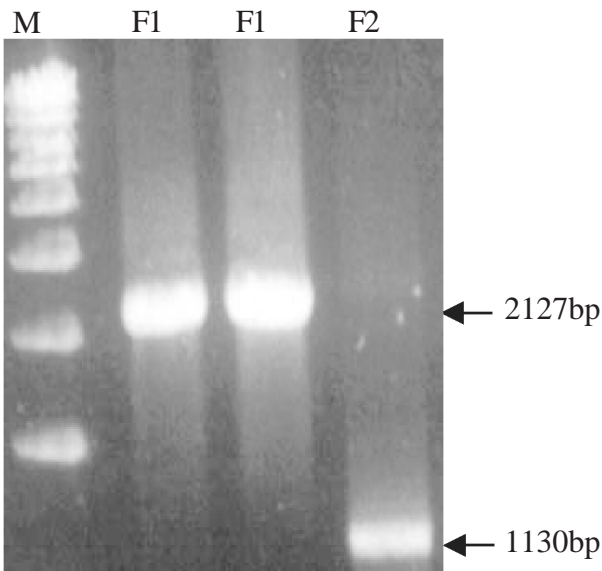


Fig. 5. PCR amplification of CYP21 gene into two fragments F1 and F2

for the purpose. CYP21P has an 8bp deletion in exon 3 which differentiates it from the active CYP21. Taking advantage of this, allele specific primers have been designed for selective amplification of active gene CYP21. Fig. 5 shows the selective amplification of active CYP21 gene in two fragments of sizes 2.1 kb and 1.1 kb respectively. These amplified fragments are now being sequenced using primers designed to screen the entire gene including coding as well as non coding

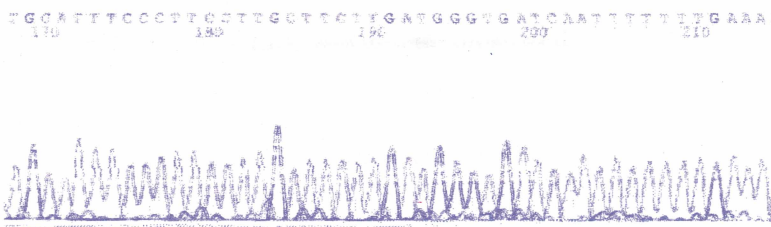


Fig. 6. Partial sequence of promoter region of CYP21 gene

sequences. Fig. 6 shows the partial sequence of promoter region of the CYP21 gene.

### Antigens involved in Ovarian Autoimmunity

In POF onset of amenorrhoea occurs in women <40 yr and is associated with hypergonadotropinism and hypogonadism. In a significant number of such idiopathic cases, POF is known to be associated with autoimmunity. Detection of specific autoantibodies

therefore remains the most practical clinical and research marker of autoimmune disease. With the development of a specific, simple, non-invasive and sensitive test, several POF patients were enrolled to screen for ovarian antibodies. Sera from 37 POF patients and 25 age-matched controls were screened. Nine of the 37 samples were positive for ovarian antibodies. Most of the sera identified multiple antigens depicting the heterogeneity of the disease. By immunohistochemistry, it was observed that there was no uniformity in the targets of attack. The targets were either ooplasm of the oocyte, interstitial region of the corpus luteum or theca cells.

### Expression of Endometrial Functional Markers in Fertile and Infertile Cases

Implantation involves a series of sequential events which culminate into the superimposition of an altered structural and molecular phenotype on the receptive endometrium. In view of this, it is highly pertinent to investigate the mechanism by which endometrium acquires an ability to accept the embryo and also the mechanism by which this receptivity is modulated in presence of an embryo. To elucidate the role of progesterone in endometrial acquisition of receptivity, comparative two-dimensional proteomics approach was employed to screen endometrial tissue proteomes of control and antiprogestin treated bonnet monkeys (*Macaca radiata*). Several proteins showing differential expression were identified in the endometrial maps of control and treated animals. Uterine flushing from control and treated animals was also compared by 2D proteomics. Uterine flushings from antiprogestin treated animals showed abundance of some proteins in the MW range of 25-15 kDa and PI range of 4-7. 2D spots corresponding to these differentially expressed proteins have been cored and analysed.

Studies initiated with the objective to investigate endometrial alterations at structural and molecular level in response to embryonic stimuli during early pregnancy in bonnet monkeys were pursued further. Endometrial tissues were collected from pregnant and non-pregnant animals on days 6 and 9 post ovulation. Remarkable spatio-temporal alterations were observed in the endometrium at histological level with the advancement in pregnancy. Endometrial insulin like growth factor binding protein 1, prolactin,

cyclooxygenase-1 and -2 showed higher expression in pregnant animals as compared to non-pregnant animals demonstrating the role of pregnancy induced stimuli in modulating the endometrial profile.

### Proteomic Approach for Assessment of Pathophysiology and Progression of Endometriosis

Studies were continued at NIRRH to understand the precise etiology and pathophysiology of

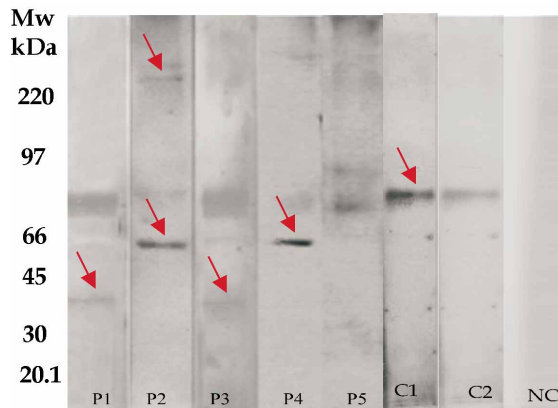


Fig. 7. Western blot showing endometrial antigens detected using sera from women with endometriosis P<sub>1</sub>-P<sub>5</sub> Endometriosis patients, C1, C2 Control, NC Negative control (secondary only control)

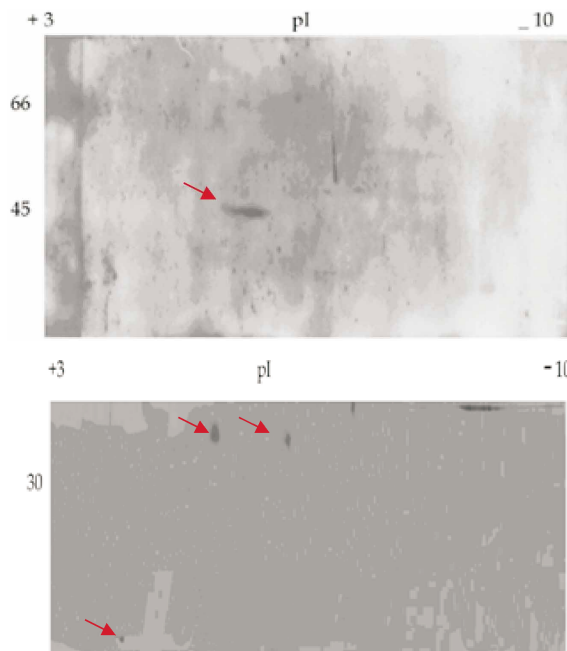


Fig. 8. (A) 2D Western blot analysis using P2 serum; (B) 2D Western blot analysis using P1 serum

endometriosis using proteomics approach. Using the sera from endometriosis patients, endometrial antigens of mol wt 30, 45, 88 and 220 kDa were detected by Western blot. To further characterize these identified antigens 2D Western blotting was carried out using the sera of patients, which had shown reactivity on 1D electrophoresis (Fig. 7 and 8). Immunohistochemistry revealed reactivity to glandular epithelium and stroma of human endometrium in secretory phase (Fig. 9). Studies are underway to ascertain the role of these antigens in the pathophysiology of endometriosis.

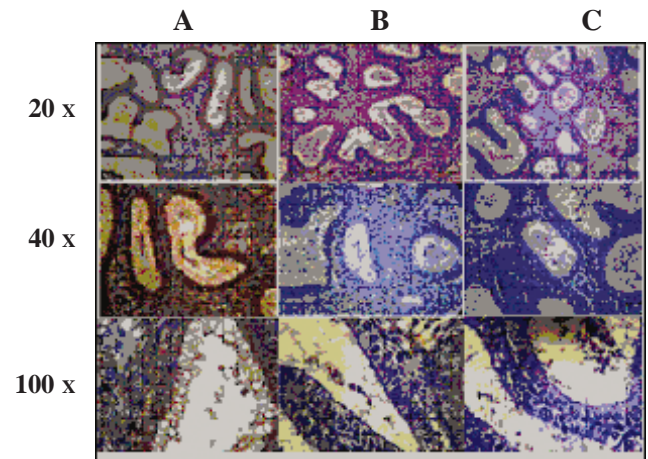


Fig. 9. Immunohistochemistry showing reactivity in glandular epithelium and stroma (P2 serum of human endometrium in secretory phase. (A) : P2 serum endometriosis serum; (B) Control serum; (C) Secondary only control.

### MALE INFERTILITY

#### Genomic Study of Y Chromosome Microdeletion in Severe Oligoasthenozoospermic and Non-obstructive Azoospermic Males

Severe impairment of spermatogenesis due to genetic factors such as chromosomal abnormalities and microdeletions of the genes on the Y chromosome are increasingly seen to be some of the causes of male infertility. These factors could affect the embryo quality. Furthermore, assisted reproductive technologies (ART) such as intra cytoplasmic sperm injection (ICSI) is known to transmit these deletions to the male offspring. It thus emphasizes the need to screen infertile men who would be potential candidates for ICSI programme.

A total of 650 infertile males (mean age 32 yr) have been screened and 54 new patients recruited, constituting a total of 180 subjects. Mean duration of trying time was 6 yr (2-11yr), 99% had primary infertility. Screening for microdeletions by 6 STS primers was carried out. Additional 5 STS primers were used for AZFa, b and c regions and 100 subjects were screened with a total of 11 STS primers. Amongst the 180 subjects, 95 were azoospermic and 85 severe oligoasthenoazoospermic.

Abnormal karyotype was seen in 4.4%. (mosaicism for Klinefelter's Syndrome in 7 and inversion of Y in 1) subjects. Microdeletions were seen in 3.48% men. No additional deletions were noted in 100 subjects where 5 more STS primers were used. Microdeletions in AZF, a and b, were predominantly seen. Phenotype/genotype correlation was not observed although phenotype picture in the patients with microdeletions showed poor fertility prognosis with reference to testicular histology which showed Sertoli cell only in 5 cases and maturation arrest at spermatocyte level in one case; while, in 36% patients without deletions, testicular histology showed hypospermatogenesis which has a better fertility prognosis during ICSI. The study so far indicates a strong need for screening and counseling of couples undergoing ICSI in view of 7.8% genetic aberrations, which could be transmitted to the male progeny.

To understand the role of the gene and protein expression and its impact on spermatogenesis resulting in a particular infertile phenotype, the mRNA of the DAZ gene was studied. It showed a normal expression on the spermatogonia to the round spermatids in a normal testicular tissue, while a weak expression in the germ cells was observed in the subject who was azoospermic, but did not have a microdeletion indicating a low expression of the DAZ gene which could be the cause of decreased spermatogenesis (Fig. 10). Interesting leads with the RBM gene, one of the two frequently deleted loci seen in this study, have been observed. In-depth characterization of the this gene and protein has been undertaken. Standardisation of the Western blot for RBM in normal human testicular

protein (which showed a size of approximately 43kDa) (Fig. 11) is in progress.

Immunolocalization was observed on the mid piece and post acrosomal region of the sperm of a fertile individual (Fig. 12). Expression of spermatogenic genes

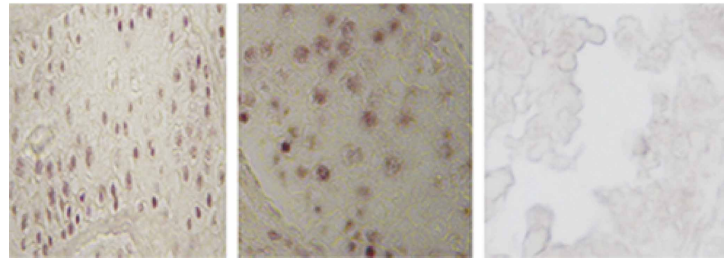


Fig.10. (A) 2D Western blot analysis using P2 serum; (B) 2D Western blot analysis using P1 serum

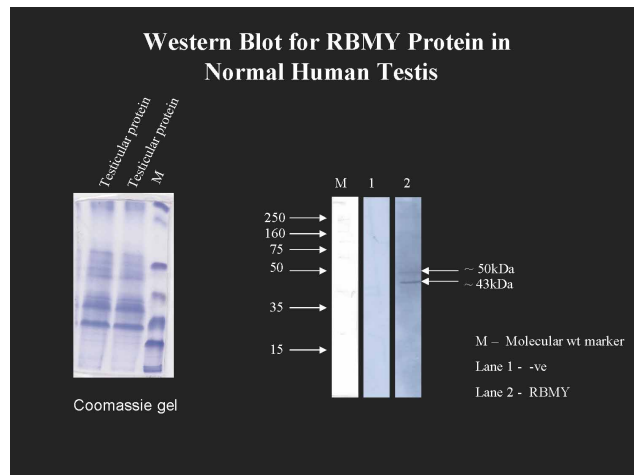


Fig. 11. Western blot for RBMY protein in normal human testis

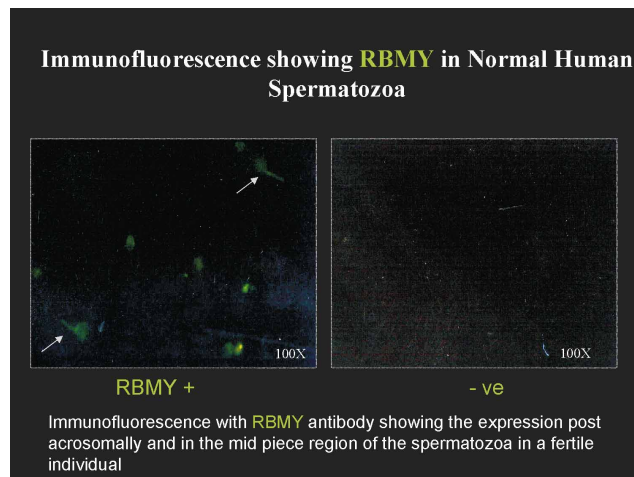


Fig. 12. Immunofluorescence showing RBMY localization in normal human spermatozoa

and their proteins, the testicular growth factors and steroid hormones are interrelated in the complex cascade of spermatogenesis and any aberration in this interaction can lead to infertility.

### Factors Modulating Spermatogenesis in Severe Oligoasthenozoospermia and Non Obstructive Azoospermia

Study was undertaken with the objectives to (i) understand the role of FSH and local secretory factors of Sertoli cells in spermatogenesis and to correlate these observations with testicular histology and (ii) to identify non-invasive markers of Sertoli cell secretory function in order to predict the potential of sperm retrievals during testicular sperm aspiration (TESA)/ICSI programmes for alleviating male infertility. Earlier observation revealed that FSH and testicular volume were not reliable indicators of spermatogenesis hence a better marker for sperm retrievals was explored. To evaluate whether serum inhibin B and FSH could be used together as indicators of spermatogenesis potentially and subsequently used as non invasive markers for sperm retrieval, 53 infertile men [(non obstructive azoospermia (29) and severe oligoazoospermia (24)] and 10 proven fertile men (controls) were recruited. The result showed higher FSH values in azoospermic men with severe testiculopathy *viz.* Sertoli cell only/maturation arrest. Inhibin B was seen to be significantly lower in this group (Fig. 13) as

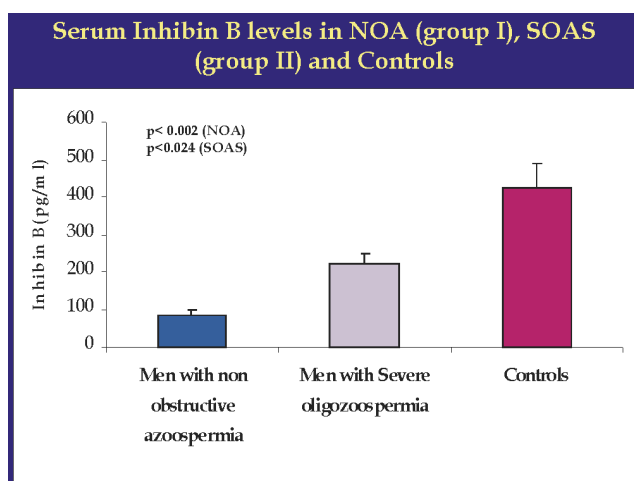


Fig. 13. Serum inhibin B levels in non-obstructive azoospermic and severe oligoazoospermic men

compared to men with severe oligoazoospermia indicating that the Sertoli cells as well as the germ cells were affected in subjects with high FSH and low inhibin secretion. Data so far suggest that when both these values are considered, the testicular pathology is more predictive and hence could be used as a non-invasive marker for sperm retrievals. The study is ongoing and more number of cases are being recruited.

### Generation of Transgenic Mouse Model of Male Infertility to Study the Molecular Mechanism of Block of Spermatogenesis

Among the genes involved in spermatogenesis, c-kit appears to play a crucial role in gonadal differentiation and formation of spermatozoa. Development of c-kit transgenic infertile mice is ongoing at NIRRH under an Indo-US project. This will help to ascertain some of the causes of infertility in men.

Sequence specific primers were designed and PCR amplified 5' untranslated region of Rouse sarcoma virus LTR (RSV-LTR) using CMV-GFP plasmid as a template and an expected size of 157bp PCR product was obtained. The product was sequenced and the sequence confirmed with data base sequence. The amplified product was cloned in pMosblue vector at EcoRV site and transformed into *E. coli* competent cells. The presence of an insert was confirmed by colony PCR using vector specific primers. C-kit gene promoter was PCR amplified using gene specific primers and an expected size of 302bp PCR product was obtained. After sequence confirmation, the product was cloned in pMosblue vector. The presence of insert has been confirmed by both colony PCR and restriction digestion.

### Spermiation Failure: A Potential Target for Contraception

Studies were carried out at NIRRH to deduce the role of estrogen in spermatogenesis. Estradiol treatment of adult male rats lead to failure of release of step 19 spermatids into the lumen in stage VIII and their retention in stages IX-XII. Effect of estradiol on cytoskeletal (F-actin, vimentin and  $\beta$ -tubulin) and junctional (N-cadherin and  $\beta$ -catenin) proteins in

seminiferous tubules in various stages of germ cell development is also being studied.

## REPRODUCTIVE TRACT INFECTIONS/SEXUALLY TRANSMITTED INFECTIONS

### Clinical Trial of Praneem

Abnormal vaginal discharge is the most commonly observed gynaecological condition for which women seek medical attention. There are multiple pathogens, which give rise to these complaints and clinical signs. A wide range of effective therapies have been tried and efficacy of some indigenous drugs is being evaluated. Praneem polyherbal tablet (PPT) developed as microbicide was tested during Phase II clinical trial for treatment of bacterial vaginosis, *Candida* and *Trichomonas* infections. A total of 141 women was included in the trial. Cure rates for bacterial vaginosis (BV), *Candida* and *T. vaginalis* (TV) were found to be 75.6%, 77.3% and 100% respectively. The data also indicated that the PPT treatment was more effective in presence of single pathogen (BV – 68.0%; *Candida* – 77.4%; TV – 100%) in comparison to cure rate in the presence of multiple pathogens [BV + *Candida* – 54.5%; BV + TV – 100.0%; *Candida* + TV – 50.0%]. Majority of the subjects (97.2%) reported symptomatic relief from the abnormal vaginal discharge. Phase III trials are planned in coming year.

### Purification and Characterization of CD4 Independent 160kDa Sperm Receptor for HIV

Studies were carried out to understand the role of spermatozoa in transmission of HIV. The modality of HIV entry into spermatozoa on which conventional CD4 receptors are absent, is not yet clear. Attempts to understand the mechanism of HIV transmission resulted in identification of a CD4 independent 160kDa HIV receptor protein on the spermatozoa for the first time. Western blot analysis of sperm proteins demonstrated specific binding of gp120 as well as cell free HIV to 160kDa protein band. Further characterization demonstrated that 160kDa HIV binding protein is distinct

from conventional CD4 receptors and binding of  $I^{125}$ gp120 to 160kDa protein can be displaced specifically by unlabelled gp120. Preliminary studies demonstrated the differential expression of this protein in sperm samples from individual donors and the samples devoid of this protein did not exhibit binding of gp120 HIV envelope glycoprotein to any protein in sperm extracts. Sequencing of the cDNA for 160kDa HIV receptor protein showed sequence homology with human mannose receptor. Efforts are on for development of modalities for the prevention of sexual transmission of HIV and to understand the mechanism of CD4 independent interaction of HIV.

## DEVELOPMENT AND EVALUATION OF NEWER MICROBICIDES

### Nisin: The Antimicrobial Peptide for the Control of Fertility and Sexually Transmitted Infections

Study to develop dual protective vaginal products having antimicrobial and spermicidal properties is ongoing at NIRRH. Antibacterial and spermicidal properties of nisin, a 34 amino acid peptide, have already been reported last year. During the year under report, to potentiate the anti-HIV activity, a cocktail of nisin with carrageenan was prepared using carbopol as a gelling agent. The study on effect of gel formulation on fertility in rabbits and on STI causing pathogens (*Chlamydia glabrata*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, *C. tropicalis*, *N.gonorrhoeae*, *Trichomonas vaginalis*, *E. coli* and *Staphylococcus aureus*) is in progress.

## DEVELOPMENT OF NATIONAL GUIDELINES

### National Guidelines for Management of Reproductive Tract Infections (including Sexually Transmitted Infections)

The Council is developing National Guidelines for Management of RTI/STIs for the purpose of incorporating in the Reproductive and Child Health

Programme. The specific objectives are to (i) conduct a rapid review of management practices (operational, clinical, laboratory) on RTI/STIs at different levels of health system in India; (ii) share assessment findings with a group of experts and evolve operational as well as quality case management guidelines for RTI/STI services; and (iii) review the existing international guidelines for RTI/STIs and prepare operational and quality case management guidelines suitable for India's health facilities.

### **National Guidelines for Assisted Reproductive Technology (ART) Clinics**

National Guidelines for Accreditation, Supervision and Regulation of ART Clinics in India, developed by the Council has been accepted by the Government after minor changes and these guidelines are in the process of translation into an act. As all ART clinics have the potential of sex selection, the Council has recommended that all the ART clinics be registered under PNNDT act immediately and when ART act/bill will be developed and implemented all the ART clinics would be again registered under ART act for the purpose of accreditation, supervision and regulation of these clinics as this provision is not present in the existing PNNDT act.

## **MATERNAL HEALTH**

### **Management and Outcome of Previous Cesarean Section at Teaching Hospitals in India**

Safety in childbirth for women with prior cesarean is a major public health concern. Repeat cesarean section and planned vaginal birth after cesarean section are both associated with benefits and harms and correct management represents one of the most significant and challenging issues in obstetric practice. Prospective data was recorded on management practices, associated complications and mortality for a period of 8 months in 2004-2005 on 15,664 consecutive cases of previous cesarean section reporting for delivery at 31 medical colleges/teaching hospitals.

A total of 1,55,863 deliveries were recorded, of which 28.1% were cesarean sections. The rate of repeat cesarean sections was 10.1%. The average age of the

women delivering by repeat cesarean section was  $26.1 \pm 3.9$  yr. Of the total deliveries, 74.5% were second para, 42% were from rural areas and 89% were booked cases. Period of gestation was less than 37 weeks in 27.2% women. Vaginal delivery carried out in 25.8% women was successful in 62.3%. Overall, 16% women had successful vaginal birth after cesarean, 47% a repeat elective and the remaining 37% had an emergency cesarean section. Major indications for repeat cesarean section included dystocia (44.2%), previous 2 cesareans, fetal distress, doubtful scar integrity, non-progress of labor, severe PIH/eclampsia and others. In majority of cases the surgical technique was conventional and only in 1.9% the Misgav-Ladach technique was used. Scar dehiscence and surgical complications were each observed in around 4% of cases. Out of 127 cases of uterine rupture, cesarean hysterectomy was performed in 38% women. Blood transfusion was given in 7.1% and post-operative complications were seen in 6%. Perinatal and maternal mortality was 26.4/1000 and 172/100,000 deliveries respectively.

### **Safety and Efficacy of Contraceptive Implant, Implanon**

Implanon, a single rod contraceptive implant made by the Dutch manufacturer Organon provides contraceptive protection for maximum of 3 yr. It contains 68 mg of etonogestrel in a ethylene vinylacetate (EVA) copolymer core, surrounded by a EVA membrane. Etonogestrel (3 keto-desogestrel) is a synthetic progesterone derived from 19-nortestosterone and is biologically active metabolite of desogestrel. The efficacy and safety profile of desogestrel containing oral contraceptives is well established. A study was initiated at 17 centres in August 2004 to evaluate the efficacy, side effects, return of fertility following discontinuation and acceptability in terms of continuation rate of Implanon. The study also assessed users' perspective about the method and the relative choice of the method when offered through cafeteria approach. Implanon was offered within 5 days of the last menstrual period, concurrent with MTP and to those women who are lactating with age of the last child more than 6 months. Women (age group 20-38 yr) with proven fertility and having at least one living child

were included in this trial. Till February 2006, 2550 women have been enrolled in the study and so far data on 2,355 women has been collected. Majority of Implanon acceptors were urban dwellers (69.3%), housewives (84.8%), literate (86.8%) and Hindu (78.2%) women. Net probabilities for discontinuation due to different reasons were computed by using life table technique. The discontinuation rate at the end of 12 months was 16.1%. The main reasons for discontinuation per 100 users are shown in Fig. 14. Less than 6 % of the acceptors gained

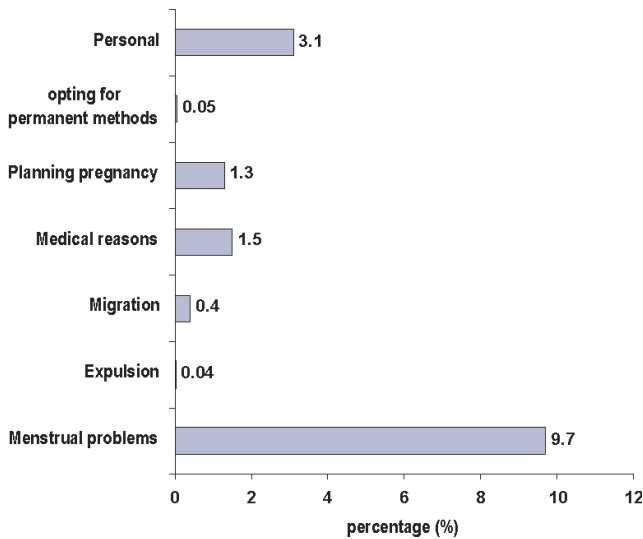


Fig. 14. Reasons for discontinuation of Implanon

more than 5 kg weight at 12 months of use of the device. Of these, only 3 women discontinued due to increase in weight of more than 5 kg. Six women experienced loss of 5 kg or more during the use of the implant. One case discontinued due to deep vein thrombosis. No method failure has been reported in this trial so far. No woman was lost to follow up. The cumulative continuation rates were 95.9, 91.8 and 83.9 per 100 users at 6, 9 and 12 months respectively. Eight hundred and thirteen women completed one year of use while 76 women have completed more than 18 months of use.

## Assessment of Prevalence of Osteoporosis in Adult Population of India

The ICMR is carrying out a task force study at four centres—AIIMS, New Delhi, NIN, Hyderabad, SGPGIMS, Lucknow and NIRRH, Mumbai to establish

peak bone mineral density (BMD) reference values for Indian men and women and to assess the prevalence of osteopenia and osteoporosis in Indian population. Preliminary analysis of data on 3642 subjects has shown that prevalence of osteopenia varied between 38.8-55.8% in males and 37.5-54.0% in females. Further, prevalence of osteoporosis was found to vary between 16.6-49.2% in males and 18.3-51.1% in females.

## CHILD HEALTH

### Home Based Management of Young Infants

The ICMR project on home based management of young infants (0-60 days) comprising care of infants at birth including resuscitation, care of normal and low birth weight babies, treatment of local infections, referral of sick infants and health education is ongoing in rural areas of five states in the country. The package of intervention is being delivered by appropriately trained village level workers - *Shishu rakshaks* (SR—a community chosen worker) in one arm and *anganwadi* workers(AWW) in second arm. Training is the most crucial activity in this project. A total of 278 SRs and 270 AWWs have been trained. Following completion of training in January 2005, independent evaluation of village level workers was carried out by Society for Education, Action and Research in Community Health (SEARCH) and ICMR for knowledge evaluation, assessment of skills, attitude and behaviour, hand washing, respiratory rate counting, weight recording, temperature measurement, detection and management of birth asphyxia and injection skill on dummies and interpersonal communication. Seventy percent SRs and 23% AWWs scored more than the cut off mark for delivery of intervention (Fig. 15). They were further provided reorientation training and reinforcement of skills during field practice. Of the SRs scoring >70%, 26 workers participated in piloting of intervention. They were provided further reorientation training and hands on practice. Fifty three probable cases of sepsis were successfully treated during piloting of intervention. Three deaths that occurred during piloting were unrelated to intervention. Results of piloting were reviewed by Data Safety Monitoring Board and Central Ethical Committee

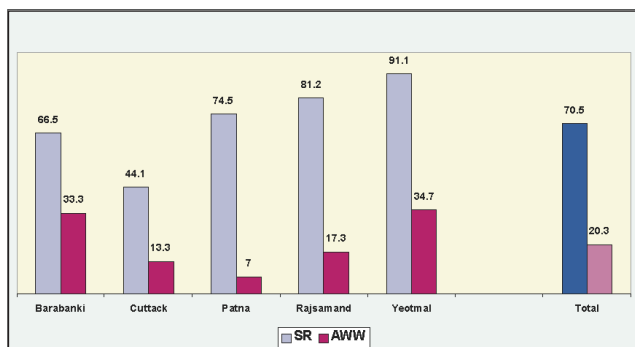


Fig. 15. Proportion of SR/AWW with score 70 & above in final evaluation

of ICMR and were found to be satisfactory. The Committee recommended scaling up of intervention.

### Effectiveness of Amoxicillin vs co-trimoxazole in the Treatment of Pneumonia in Children

Acute respiratory infection (ARI), mostly pneumonia, is one of the leading causes of death in younger children in developing countries. The Council carried out a multicentre study in 7 districts - Delhi, Nagpur, Trivandrum, Mumbai, Lucknow, Bhopal and Chandigarh at 14 rural primary health centres to study the effectiveness of 3 day amoxicillin vs 5 day co-trimoxazole in the treatment of non-severe pneumonia in children aged 2-59 months of age. Data collection has been completed at all the centres in April, 2005. Final analysis of data is ongoing.

## ADOLESCENT REPRODUCTIVE HEALTH

### Knowledge and Practices of Adolescent Girls

Adolescents constitute one-fifths of India's population and yet their sexual health needs remain largely unaddressed in the national programme. The Council supported a community survey to study knowledge and practices of adolescent girls regarding reproductive health with special emphasis on hygiene during menstruation in 50 villages selected from 5 districts namely Kamrup, South-West Delhi, Mysore, Dhar and Barabanki. A total of 500 adolescent girls, 500

mothers of adolescent girls, 93 women opinion leaders and 150 women ICDS and health functionaries were interviewed using field tested research tools. It was observed that 93.6% girls were literate but only 49.2% went to school and 41% had no access to toilet facilities. The mean age of menarche was 12 yr. Most girls (70.6%) were not aware of menstruation till onset of menarche and had limited knowledge of physical changes during puberty. The main source of information was mother(37.6%), siblings(32.8%), and friends(27.6%) (Fig. 16). The onset of menarche caused fear(62.3%) and shock (43.9%) in them. Many mothers of adolescent girls considered menses as dirty and polluting (70.4%) but performed 'no ceremony' for menarche (56.8%). Common complaints of adolescent girls regarding menstruation included vaginal discharge, itching, smelling discharge and painful periods (Fig. 17). They

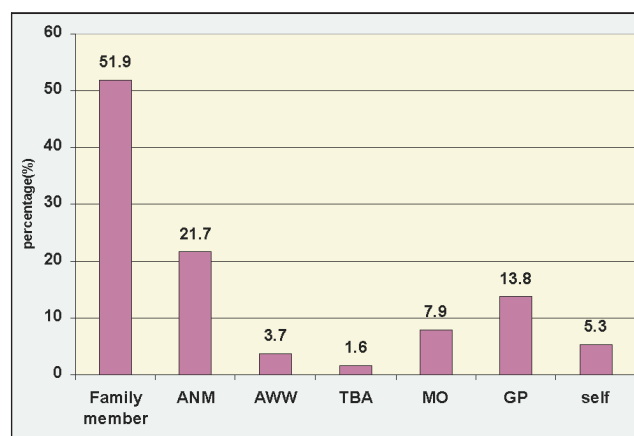


Fig. 16. Health seeking behaviour of adolescents with vaginal discharge

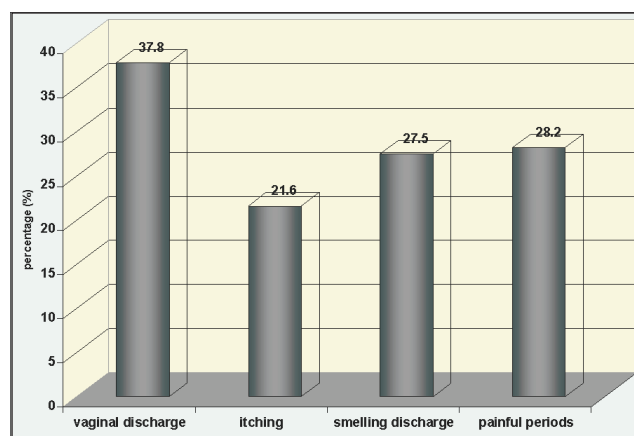


Fig. 17. Common complaints among adolescents

restricted movement of the girls within house (68%) and restrained them from consuming sour food. Nearly 57.6% girls and 74.1% mothers reused cloth after washing the menstrual discharge. It was found that knowledge of HIV/AIDS/RTIs/STIs was incomplete in all respondents (Table 1). Even though 85% girls, 69.6% mothers and 83.8% women opinion leaders had heard about HIV/AIDS, their knowledge about mode of transmission and prevention was limited. There is need of a multi-pronged strategy using all kinds of media to disseminate information on reproductive health of adolescent girls/women with special emphasis on hygiene during menstruation.

**Table 1. Knowledge of HIV/AIDS among adolescent girls (n=500) multiple response**

Mode of transmission (%)	Prevention of HIV/AIDS (%)
Unprotected sex 46.6	Safe sex practices-37.8
Infected blood transfusion (31.8)	Safe blood transfusion (23.6)
Infected needle /syringes/	Using sterilized

### Improving Service Utilization by Adolescents through Urban Health Posts in Mumbai

A project has been initiated with the aim to create an adolescent friendly environment at the Urban Health Posts as well as in the community and improve health service utilization by adolescents through networking within the existing health care infrastructure. A comprehensive adolescent health care program has been conceptualized in collaboration with Municipal Corporation of Mumbai service units to meet the multiple needs of adolescents, particularly for contraception and prevention of STIs. Study will be conducted in three phases—(i) preparatory phase of six months with situational analysis of the needs and perceptions of adolescents and identification of the partner institution with facilities to offer reproductive health services, (ii) intervention phase of two years for identification of adolescent related health issues and providing services and (iii) the third phase of six months

for process, outcome measurement and preparation of the report.

The intervention phase of the project is ongoing. Following interventions have been done (i) Adolescent Friendly Centres were named as *JAGRUTI*, through a participatory approach. They were inaugurated in February, 2006 amidst a gathering of adolescents and local committee members and other stake holders. Information on (i) availability (ii) dissemination of services being provided at the centre was disseminated through pamphlets, posters and advertisement through cable TV. A number of non-health related activities were planned such as essay and drawing competitions to build rapport and attract the adolescents to the centre. (iii) meetings were held with parents, teachers and gatekeepers to build rapport and seek support in provision of sexual and reproductive health services to the adolescents.

The following output of interventions have been observed: (i) married adolescent women have started approaching the centre for family planning methods, pregnancy detection, complaints of vaginal discharge, menstrual complaints, infertility and other general health complaints, (ii) unmarried adolescent girls approached the centre for premarital counseling, menstrual complaints and queries on sexual and reproductive health, (iii) adolescent boys approached the centre mainly to procure condoms and also for information on a number of their sexual and reproductive health problems and (iv) older women also approached the centre for hypertension, arthritis or other general health complaints but to a lesser extent.

### Evolving a Model for Improving Reproductive Health among Rural College Youth in Maharashtra

The ongoing study is based on developing and applying information, education, communication (IEC) and counseling intervention backed up by referral linkages with the district health services. The main objective of the study is to develop a replicable and sustainable model for provision of sexual and reproductive health services to college-based youth in Thane district. The ongoing study is of 30 months duration and will have an experimental design conducted in three phases.

In order to assess the awareness and views about reproductive health as well as sexual behaviour of rural college youth, baseline data (qualitative and quantitative) have been collected. A self-administered, semi-structured questionnaire was used for survey, which included 1500 students (800 male and 700 female) in the age group 15-24 yr.

The following interventional activities were carried out during January to December 2005 in four experimental rural colleges. A total of 65 IEC and peer leader training programs were conducted throughout the year and 7795 students (4184 boys; 3611 girls) attended these programs. The subjects included were human physiology, conception, menstruation, masturbation, contraception, RTIs, STIs and HIV/AIDS. Counselling centres were started in all the four experimental colleges in the month of July 2005. Trained counsellors made service provision available once a week for at least 5 h in each college. A total of 776 students (311 boys and 465 girls) attended these counselling centres in 5 months. IEC material (various books and pamphlets) collected from a number of organizations was made available at the college library for teachers and students for reference. A total of 1200

books, pamphlets on reproductive and sexual health of youth have been distributed among peer leaders, teachers and librarians. Orientation programs were conducted in colleges for all the teaching and non-teaching staff. A total of 20 male and female teachers from the colleges (average 5 teachers) took initiative on their own for further training.

Competitions such as essay writing, slogans, making poster and debates were organized in all experimental colleges. Students from NSS were provided with detailed information about 'peer educator' activity. Majority of the participants of the various competitions and NSS activity have opted for further detailed training as 'peer educators'. Students were also told about the 'peer educator' programme through regular IEC programmes in respective colleges. A total of 200 students (average 50 boys and girls from each college) were selected and provided with 'peer educator' training. Government health care providers (block level) have been oriented and reproductive health service provision was made available for students from experimental colleges at PHCs. Post survey in all colleges has been initiated in the month of December 2005.