

Reproductive Health

Research in the area of reproductive health is being pursued by the National Institute for Research in Reproductive Health (NIRRH) located at Mumbai and the Human Reproduction Research Centres (HRRC) of the Council.

At NIRRH, several new epididymal testicular proteins with role in sperm maturation and function have been identified and characterized. Molecules with dual roles of contraceptive and anti-microbial activity have been purified from the hemolymph of Indian mud crab, *Scylla serrata* and are being assessed. Nisin, a food preservative has been demonstrated for the first time to also have antifertility activity.

An HIV receptor/binding protein on spermatozoa (a 160 kDa sperm protein that is bound specifically by gp120 HIV envelop glycoprotein as well as cell free HIV) was identified by Western blot analysis suggesting an alternative mode of HIV entry into spermatozoa which is devoid of conventional CD4 receptors.

Reproductive health programme for adolescents has been initiated by the Institute. The knowledge, attitudes and need of the adolescents are being studied and a programme for their long term need will be provided.

FERTILITY REGULATION

BASIC RESEARCH

Identification and Characterization of Epididymal Antigens

(i) Using neonatal tolerization

Sperm antigens play a crucial role in reproduction and these proteins are mostly acquired either from the testis or the epididymis. Neonatal tolerization was used for raising monoclonal antibodies to rare or minor antigens. Monoclonal antibodies so generated as probes using epididymis specific antigens were identified. One of the five epididymis specific monoclonal antibodies obtained was

characterized. Immunochemical characterization of the cognate protein revealed a tissue specific and cell type specific pattern (Fig. 1).

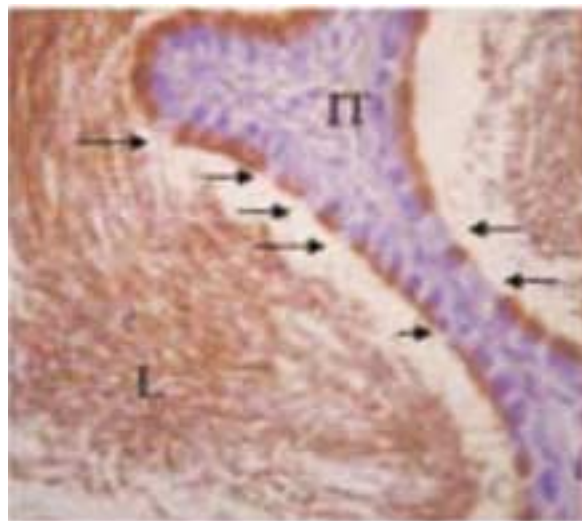


Fig. 1. Tissue and cell type specific localization of the protein.

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

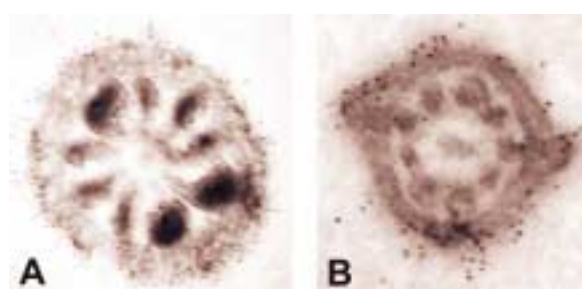


Fig. 2. Immunogold labelling of the protein on the midpiece (A) and sperm tail (B) using antisperm antibodies.



(ii) Using antisperm antibodies

Using vasectomised mouse model, monoclonal antibodies were generated for use as tools to identify and characterize functionally relevant and conserved testicular and epididymal proteins. One of the monoclonal antibodies D5E5 identified a ~70kDa testis specific antigen (Fig. 3).

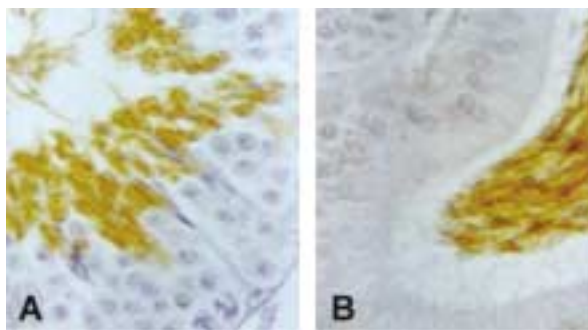


Fig. 3. Immunohistochemical localization of the protein in mouse testis (A) and epididymis (B).

The cognate antigen is expressed post-meiotically in a stage specific manner starting from elongating spermatids at stage 8 of spermiogenesis upto mature spermatozoa. Immunofluorescence (IIF) studies showed that the antigen is localized on the tip of acrosome as well as principal piece of tail (Fig. 4). The antigen is conserved across the species on rat, bull, marmoset and human spermatozoa as seen by IIF and exhibits species specific domain localization.

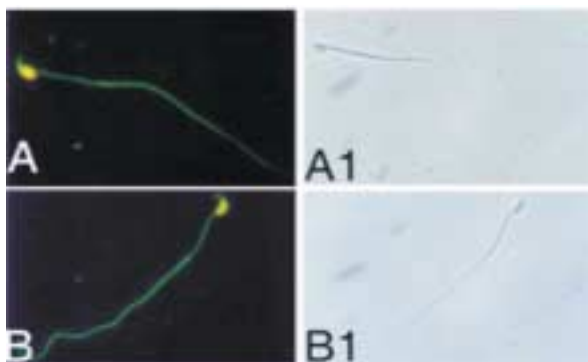


Fig. 4. Immunofluorescent localization on the mouse testicular (A) and epididymal sperm (B). A1 and B1 are corresponding phase contrast images.

Its testis specificity, acrosome and tail localization suggest an important role in reproduction.

Studies with 80 kDa Human Sperm Antigen and its Synthetic Peptides

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

Passive immunization of rats with 10 or 40 µg of the immunoglobulin fraction of antibodies to NT and peptide 1 resulted in inhibition of pregnancy in 45-70 % of the animals, respectively. In immunized rats, the epididymal spermatozoa were found to be agglutinated and antibodies were localized in the epididymis and not in the testis suggesting that antibodies do not cross the blood testis barrier.

Studies also indicate that in the epididymis the protein appears from day 40 onwards, suggesting that it may be androgen regulated.

Role of a Novel Androgen Regulated Hox-B2 containing Gene Expressed in the Epididymis

A partial sequence showing homology to the conserved region of *Hox-B2* was identified from a monkey epididymal library. In an attempt to identify epididymal proteins which play a functional role in sperm maturation, a clone was identified from the monkey cDNA library using monoclonal antibody raised against washed human spermatozoa with sequence homology to the conserved region of *Hox-B2*. The gene was expressed in adult rat, monkey and human epididymis and not in immature rat epididymis, suggesting that it is a conserved protein and is regulated by androgens. Both in the castrated as well as ethane dimethane sulfonate (EDS) treated rat model, the protein expression in the epididymis disappeared and following



supplementation with testosterone was restored to near normal levels. In developing rats, the protein was expressed from day 40 onwards, concomitant with the secretion of testosterone, also confirming its androgen regulation. The protein was expressed only in the epididymis with maximal expression in the cauda epididymis. Northern blot analysis revealed a single transcript in the monkey cauda epididymis. Attempts to obtain a full length sequence using 5'RACE resulted in a 900 bp upstream region, which has been cloned into the pGEM-T vector and is currently being sequenced.

Regulation of Endometrial Apoptosis

The human endometrium is characterized by cyclic appearances of proliferative and apoptotic changes in a steroid dependent manner. However, the precise role of steroid hormones associated with these changes remains unclear. In studies done at NIRRH, Mumbai, the female common marmoset (*Callithrix jacchus*) has been utilized as a model to study the endometrial changes in response to steroid hormones. Studies were carried out in normally cycling and ovariectomized females treated with graded doses of estradiol and progesterone. Using the TUNEL method, apoptosis was observed in the endometrial samples collected during the mid-luteal phase of the normal menstrual cycle. Expression of Bax, an apoptotic protein, was coincident with TUNEL and optimum plasma progesterone levels. In simulated cycles, the number of apoptotic and Bax positive cells was higher in the estradiol and progesterone treated group as compared to that in the estradiol alone or progesterone alone treated groups (Fig. 5). Thus, programmed cell death in the endometrium of common marmosets occurs during the mid-luteal phase and is regulated by estrogen, progesterone and Bax protein.

Role of FSH Binding Inhibitor in Ovarian Pathophysiology

The FSH binding inhibitor (FSHBI) is an intraovarian peptide of molecular weight < 4 kDa that inhibits FSH binding to granulosa cells *in vitro*. *In vivo*, the peptide induces atresia in

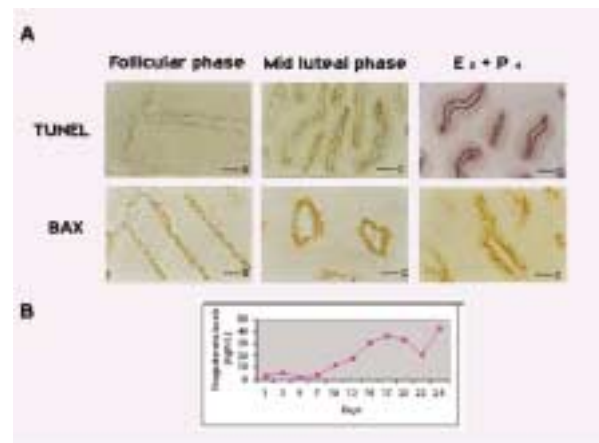


Fig. 5. (A) Detection of apoptosis by TUNEL and expression of Bax in marmoset endometrium during follicular phase, mid luteal phase and in the endometrium of ovariectomized marmoset after treatment with oestradiol (E) and progesterone (P). (B) Progesterone levels in marmosets during ovarian cycle.

mice and impairs fertility in marmosets. The N – terminal 8-amino acid fragment (Octapeptide, OP) of FSHBI exerts antifertility effects similar to that observed with the native peptide. In an attempt to study the expression and levels of OP/FSH BI in ovarian follicles, rabbit polyclonal antibodies were raised against OP and its titre assessed by ELISA. The antiserum was used for localization of OP/FSHBI in mouse ovarian follicles. While OP/FSHBI was expressed specifically in granulosa cells, the expression was higher in atretic follicles. However, no localization was noted in other mouse tissues such as liver, kidney, spleen, adrenal and uterus (Fig. 6).

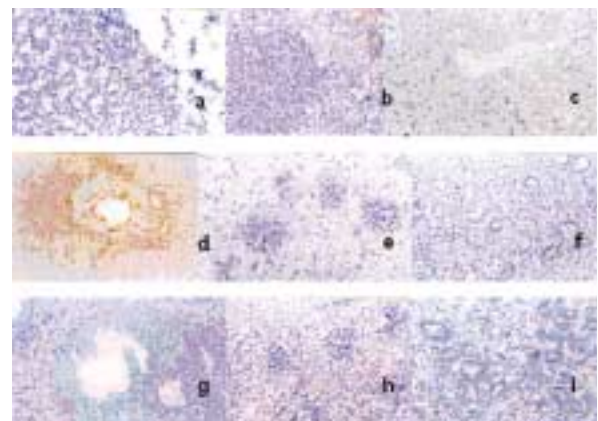


Fig. 6. Localisation of OP in mouse tissues: (a) adrenal, (b) spleen, (c) liver, (d,g) ovary, (e,h) kidney, (f,i) uterus; (a-f)-OP; (g-i) NRS control.

Oocyte-Granulosa Cell Interaction during Early Folliculogenesis

The paracrine factors secreted by oocytes and somatic cells possibly regulate many of the events of early follicular development in mammals. However, factors responsible for stimulating follicles for differentiation and development are not well defined. A study was undertaken to delineate these factors using a mouse model. Morphology of the ovary and immunohistochemical localization of growth differentiation factor-9 (GDF-9), proliferating cell nuclear antigen (PCNA) and apoptosis were studied on days 2, 4 and 6 in neonatal ovaries.

GDF-9 was located in oocytes of primary follicles while, PCNA was expressed in granulosa

cells of secondary follicles. Apoptosis, as revealed by TUNEL technique, was not observed during early folliculogenesis (Fig. 7). These studies suggest that GDF-9 secreted by oocytes of primary follicles possibly interacts with granulosa cells to induce proliferation in these cells. Studies are also in progress to understand the role of growth factors in regulation of early folliculogenesis.

Structure-Function Studies on Gonadotropins and their Receptors

Gonadotropins play a critical role in both male and female reproduction. They bind to specific high affinity receptors present on the plasma membrane of respective gonadal cells.

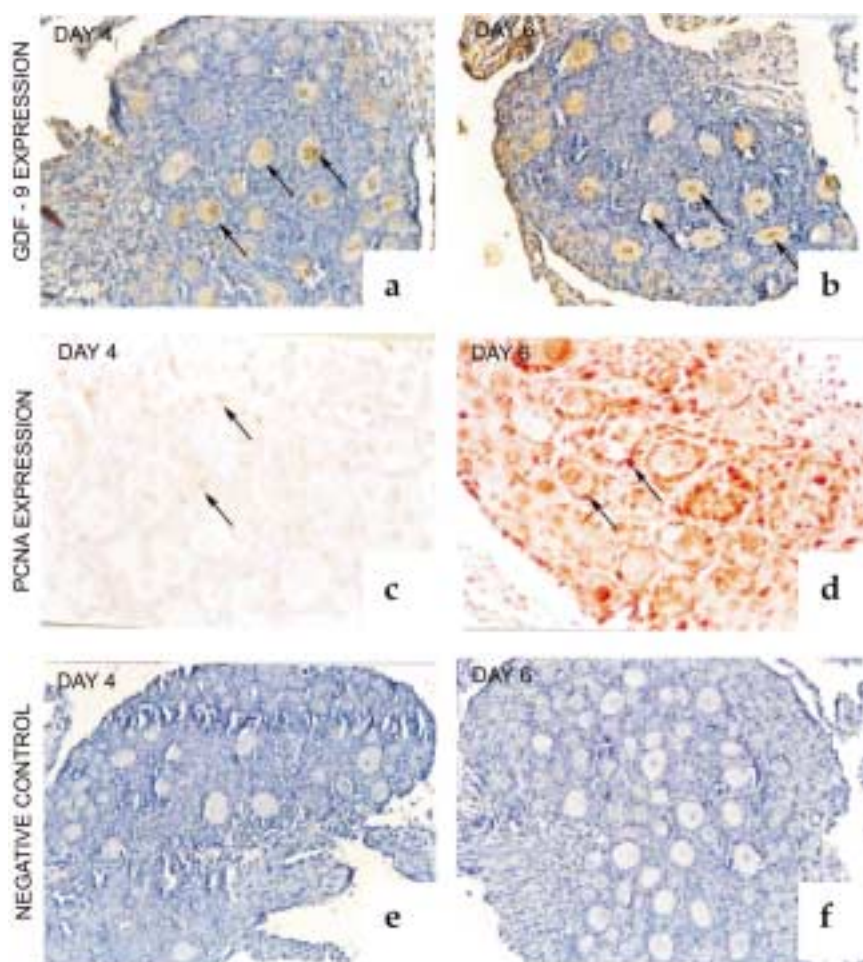


Fig. 7. Immunohistochemical localization of GDF-9 (a,b) and PCNA (c,d) in oocytes of primordial follicles in day 4 and day 6 mouse ovaries. GDF-9 is expressed in oocytes of primary follicles (a) and preantral follicles (b). PCNA's expression in granulosa cells of primary follicles (c) and preantral follicles (d). Negative control (e,f).

Understanding the structural basis of this interaction would facilitate the designing of small molecules exhibiting gonadotropin-antagonistic activities. Ten different synthetic peptides corresponding to the probable surface oriented regions of extracellular domain of FSH receptor (FSHR) were studied for their ability to modulate FSH induced cAMP levels in an *in vitro* FSH bioassay. Of these peptides 15-31, 79-89, 184-195, 216-235 and 285-300 exhibited an inhibitory effect indicating their involvement in signal transduction. Anti-peptide antibodies raised to these peptides also inhibited the binding of hormone to its receptor indicating their ability to recognize the corresponding region on the receptor surface. Ability of the anti-peptide antibodies to bind and neutralize the receptor function are being studied in depth.

CLINICAL RESEARCH

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

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

Study was undertaken: (i) to assess user acceptability and continuation rates of NET-EN; (ii) to evaluate the incidence of menstrual irregularities and other side effects; (iii) to assess the socio-behavioural aspects with respect to socio-economic and cultural diversity and (iv) study return of fertility in eligible women. The main emphasis of this study is on good counselling by qualified and trained staff in an attempt to ensure better continuation rates.

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

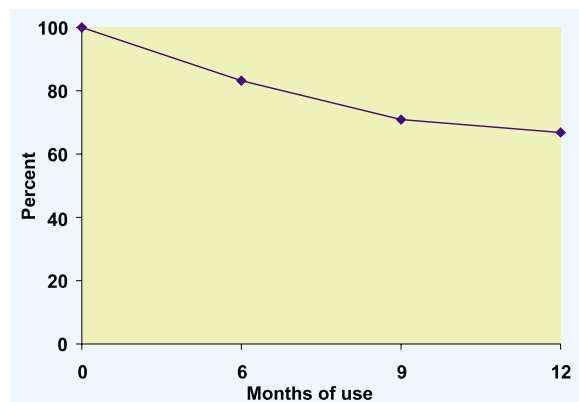


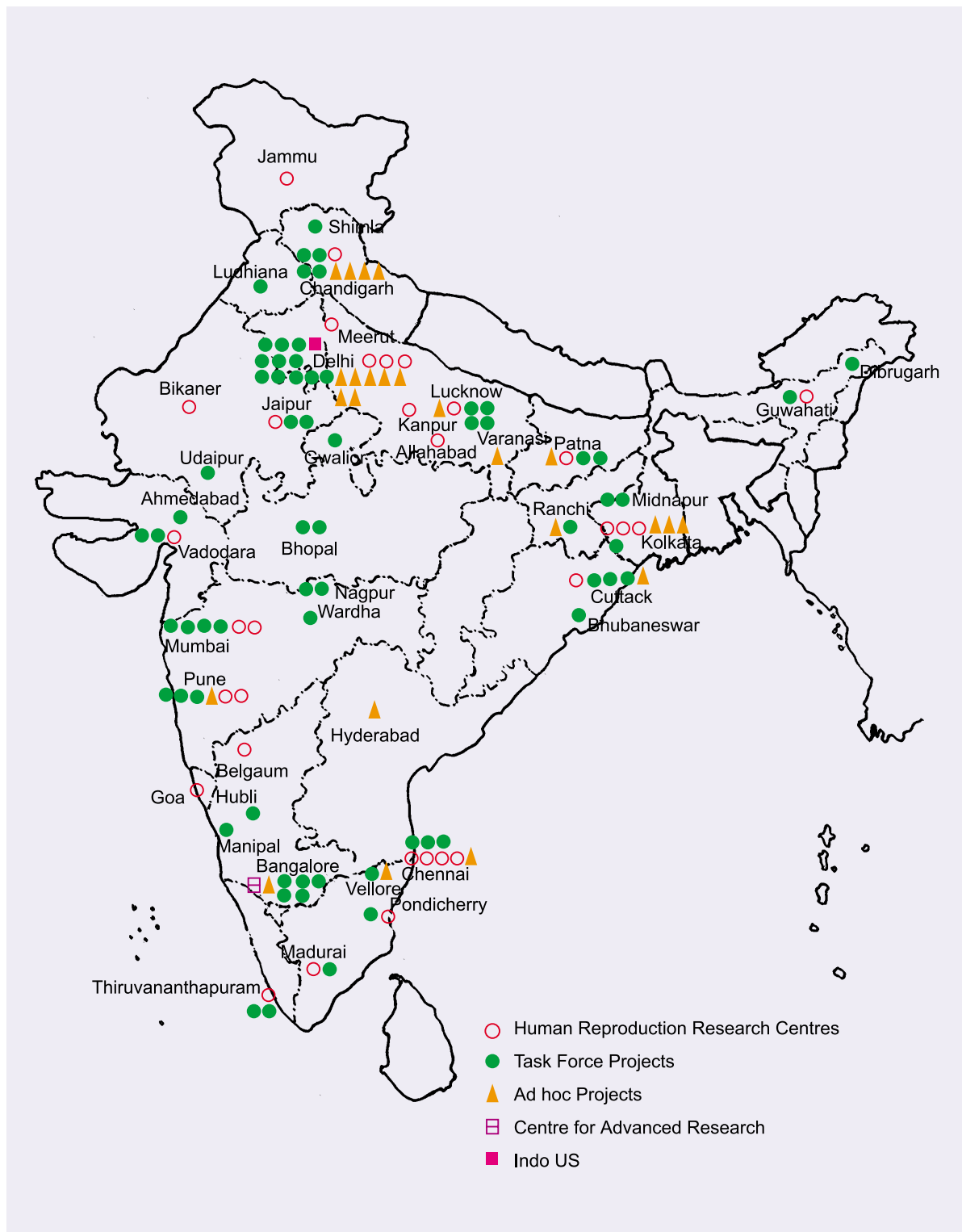
Fig. 8. Cumulative continuation rate of NET-EN.

rate of 83.3% and 67.1% at 6 and 12 months of use respectively (Fig. 8).

Improving Male Involvement in Family Welfare

In India, men play a dominant and many a times a decisive role in regulating women's access to reproductive health. In an attempt to: (i) understand family planning knowledge, perception, attitude and practices of men; (ii) plan appropriate intervention strategies for enhancing male involvement; and (iii) evaluate the impact of the interventions on the reproductive health care of the family, a study was designed to include situational analysis in both control and experimental areas; intervention phase to increase awareness and knowledge regarding reproductive health issues and services in experimental area; and impact evaluation after three years in both the areas. Analysis of the impact analysis revealed that the knowledge of correct use of condom (14.1%), withdrawal (27.6%) and rhythm (27.5%) had increased significantly (Fig. 9) and the knowledge of condom use for dual purpose *i.e.* in prevention of pregnancy and infection had increased by 6.3% in experimental area. Inter-spouse communication with regard to obtaining family planning information increased by 5.4%, permanent methods by 3.9% and spacing methods by 0.6% in experimental area (Fig.10). Overall contraceptive use had increased by 1% in control and 17.1% in experimental area (Fig.11). Female sterilization was widely accepted in control area, while condom use was higher in the experimental area.





MAJOR ICMR RESEARCH PROJECTS IN REPRODUCTIVE HEALTH



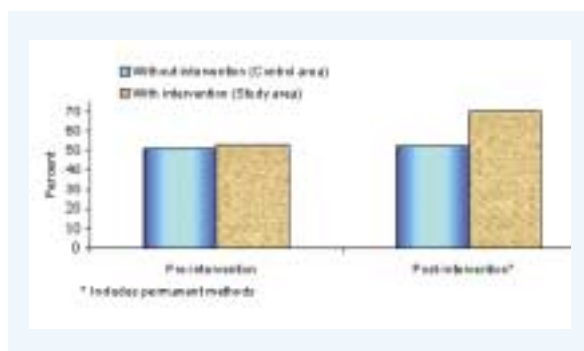


Fig. 9. Impact: knowledge of correct use of contraceptive methods.

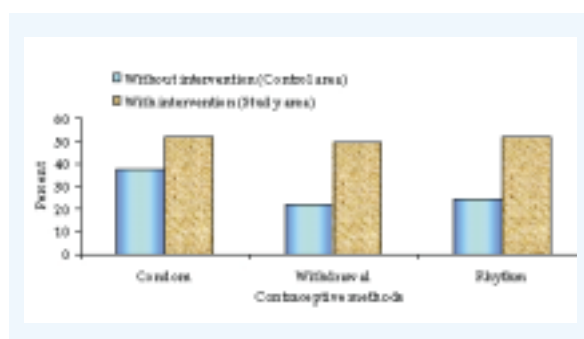


Fig. 10. Impact: Inter-spouse communication.

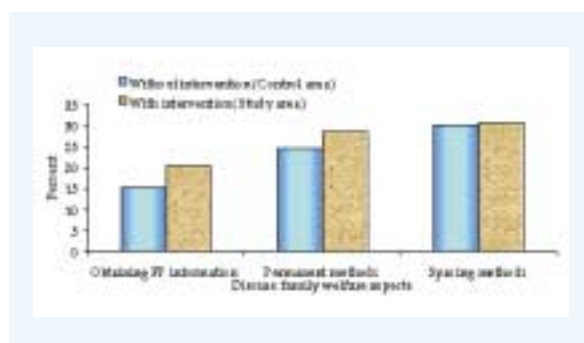


Fig. 11. Contraceptive use by couples.

Emergency Contraception with CuT 200B IUCD and Levonorgestrel

No contraceptive by itself is perfect in its use or efficacy. Therefore, so long the search for an ideal contraceptive continues there will be need for a second chance for protection against pregnancy. Lack of access to emergency contraception may subject women to unsafe abortions, which contribute significantly to maternal mortality and morbidity. Yuzpe

regimen and levonorgestrel (LNG) 0.75 mg two doses are the most widely researched and available methods besides IUCD CuT 200B.

Women requesting for emergency contraception after a single act of unprotected intercourse in the current menstrual cycle were offered as per their request 0.75 mg LNG (two doses) or IUCD Cu T 200 B if seeking within 72 and 120 hr of unprotected intercourse respectively. Women were advised to abstain or use barrier methods till the onset of next menstrual period. Of a total of 1868 women who came to the clinic seeking EC, only 37.0% could be offered. Rest of them could not be offered due to various reasons *viz.* came after stipulated date (29.9%), overdue/pregnant at the time of seeking EC (33.2%), more than one unprotected act in the current cycle (14.4%) and women who did not meet eligibility criteria for the study (22.6%). A total of 648 women accepted EC—63.6% accepted LNG and 36% Cu T 200B. Among the women enrolled for EC, 69.8% women sought EC (either LNG or IUCD) within 48 hr of unprotected act.

Women sought EC mainly (38-58%) for non-use of any family planning method, 15-23% for condom breakage/slippage, 10-12% forgot to use condom and 4 - 11% for IUCD expulsion/displacement. Forced sex was the reason given by only 1.7% women (Fig. 12).

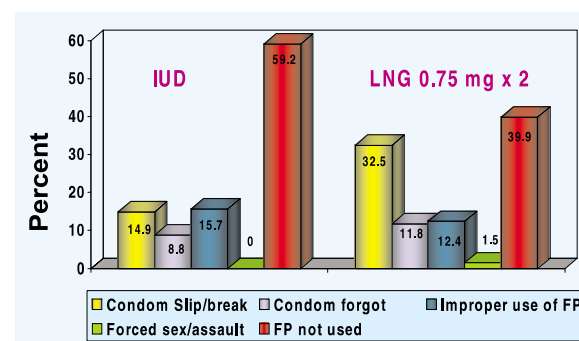


Fig. 12. Reasons for women seeking EC.

Side effects with LNG were nausea, headache and dizziness (10-14%) and with IUD low abdominal pain (49.5%) and irregular bleeding (15.2%).

Seven pregnancies were reported in the levonorgestrel group (3 user failure and 3 method failure) giving a typical use failure rate of 1.73%



and the per cent pregnancies averted with LNG were 78.7. No pregnancy was reported with IUCD CuT 200B.

Return of menstruation within 7 days of expected period was observed in 92-95% women while in 3-6% the cycles occurred earlier than one week and in 2% they were prolonged beyond 8 days.

Women's perceptions indicated that majority (93.2%) would like to consult a doctor and preferred to avail the method either from clinics/hospitals (58.9%) or on the prescription of a doctor (46.6%). Only 7.0% women preferred to avail from the chemist directly. Majority (77.8%) indicated that they would like to avail the method as and when they need it.

Clinical Trial with Once a Month Combined Injectable Contraceptive-Cyclofem (Medroxy progesterone Acetate MPA 25 mg + Oestradiol Cypionate 5 mg)

In this study, till date a total of 1330 women have been enrolled at 16 HRRCs and observed for 11387 women months of use. Of them 574 have completed one year of use. No method failure has been reported till date.

The continuation rates at 6 and 12 months were 74.2 and 68.6 per 100 users. Women discontinued the method due to various reasons which include pain at injection site, menstrual disturbances, medical reasons, planning pregnancy, use of permanent method/other FP methods and personal reasons.

Analysis of menstrual pattern for a reference period of 3 months (90 days) each indicated that 29.2% women had acceptable menstrual pattern in the first reference period which improved to 37.8% by the fourth reference period *i.e.* one year of use (Fig. 13).

Clinical Trial with SMA, an Intravasal Injectable Male Contraceptive

A novel male contraceptive approach, reversible inhibition of sperm under guidance (RISUG), was developed at IIT, New Delhi. RISUG was injected in 141 subjects at three centres in Delhi out of which two could not

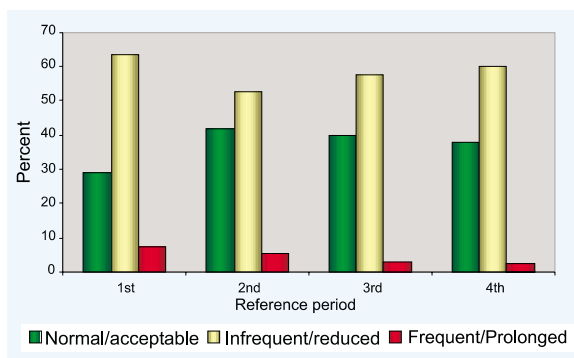


Fig. 13. Analysis of menstrual pattern of women.

complete the protocol. Therefore, 7139 subjects who completed six months of follow up were included in the analysis. Scrotal swelling was seen in 84.1% subjects at one week follow up which continued in 52.2% subjects up to one month. Around 36% subjects felt persistent pain in scrotal region, which persisted in 25.2% subjects for one month. Scrotal nodules were observed in 23.4% subjects at one week and 33.3% subjects after one months of injection. Scrotal abscess, fluid collection around scrotum and inflammatory pathology were seen in one case each after one month of treatment. Albumin and sugar were noticed in urine of 14 and one subjects respectively after six months of injection.

Outcome of the treatment was considered successful if, a) azoospermia manifested within two months of the treatment, b) three monthly semen analysis thereafter showed azoospermia and c) no pregnancy was attributable to a period following the onset of azoospermia. The data indicated that 79.1% subjects achieved azoospermia after two months of injection while rest of them achieved it between 3 to 6 months (Fig. 14). Six subjects could not achieve

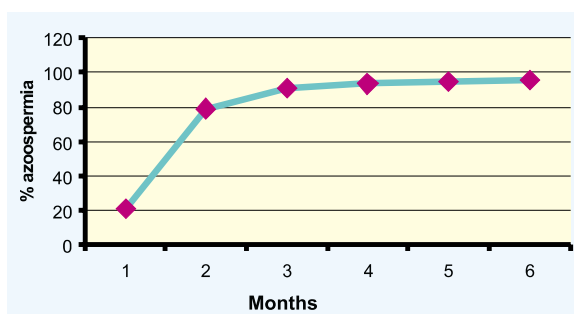


Fig. 14. Contraceptive efficacy of RISUG.

azoospermia, out of which in one subject it resulted in pregnancy.

INFERTILITY

FEMALE INFERTILITY

Studies on Genetic Aspects of Polycystic Ovary Syndrome

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

Subjects included in the study were those with documented evidence of PCOS in association with hyperandrogenicity with or

without obesity. Those with evidence of thyroid, adrenal or pituitary disease were excluded. First degree relatives of index cases along with healthy controls were also screened. The entire coding sequence of both the genes *viz.* CYP11A1 and leptin were analyzed for reported as well as novel mutations by PCR-SSCP/RFLP for each of the exons followed by DNA sequencing to confirm the detected mutations. Fig. 15 shows PCR amplification of the coding sequences of CYP11A1. Some of the PCR amplified fragments were further subjected to SSCP analysis for detection of mutations / polymorphisms. Fig.16 shows the SSCP

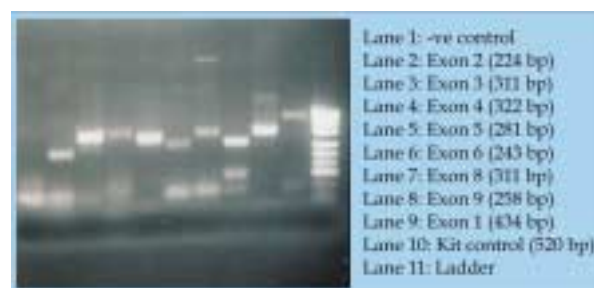


Fig. 15. PCR amplification of coding regions of CYP11A1.

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

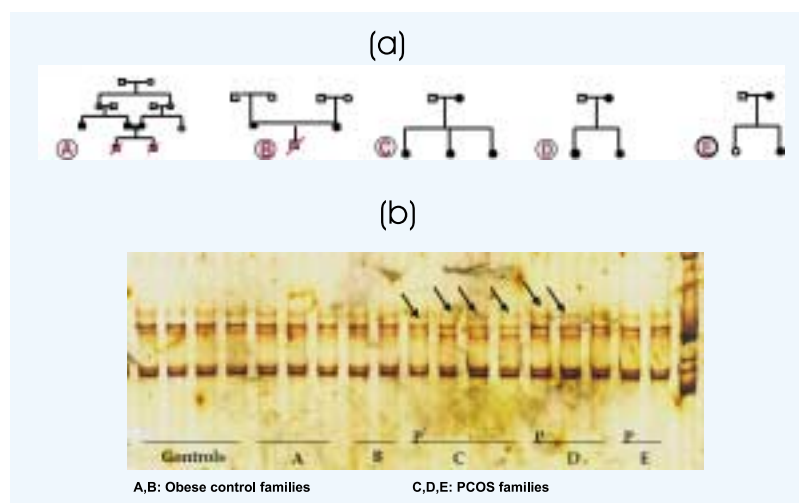


Fig. 16. (a) Pedigree charts of obese and PCOS families. (b) SSCP analysis of exon 9 of CYP11A1 gene.

Genomic Studies in Women with Premature Ovarian Failure

Premature ovarian failure (POF) defined as ovarian failure occurring in women under the age of 40 yr is being increasingly diagnosed. Several etiological factors have been postulated for early depletion of oocytes. Candidate genes or loci that have been suggested to cause familial or sporadic POF include genes on the X chromosome (POF1, POF2, FMR1), inhibin and FOX L2. Data available so far are based on Western population and there is a need to investigate this aspect in the Indian context. Study was initiated to determine the mutations/premutations in the FMR1, inhibin and FOX L2 genes in women with idiopathic POF and their families and to select the best candidate gene for determining predisposition to POF. Studies in 30 patients revealed that FOX L2 gene mutations could be one of the factors associated with early ovarian failure.

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

In an attempt to identify novel endometrial factors that may play a potential role in endometrial receptivity and implantation, differential display RT-PCR (DD-RT-PCR) approach was used to screen endometria from fertile, infertile and mated bonnet monkeys. Three cDNA fragments have been cloned that show differential pattern of expression in onapristone-treated infertile animals as compared to control animals. One of these fragments, (DD2A) was found to be over-expressed in the endometria of onapristone treated infertile bonnet monkeys and was identified to be a Rab coupling protein (RCP) by *in silico* analysis. This is the first report demonstrating existence of RCP in the endometrium and its possible regulation by progesterone.

MALE INFERTILITY

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

Microdeletions of Y-chromosome are associated with disruption of spermatogenesis.

Chromosomal abnormalities and microdeletions of Y-chromosome contribute towards poor embryo quality and early pregnancy loss.

A study has been initiated to assess the frequency and type of microdeletions associated with non-obstructive azoospermia and severe asthenozoospermia.

Sixty-nine infertile men (age 32 yr) have been recruited from the Institute's Male Infertility Clinic. Using 6 STS primers to amplify the PCR products, microdeletions of mixed type were seen in 6 subjects showing, a frequency rate of 8.6%. Further studies are ongoing.

Molecular Characterization of Human Sperm Progesterone Receptor

Classical steroid hormone receptors are transcription factors that act via genomic mode of action. However, progesterone receptors (PR) on the human spermatozoa act through a non-genomic mode of action. Study was conducted for identifying and characterizing the membrane bound sperm PR and understanding its mechanism of action.

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

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

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

On screening a human epididymal cDNA library with a 57kDa antibody, a 1.8 kb cDNA clone was identified. The sequence of this clone showed 98% homology with human chromosome



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

REPRODUCTIVE TRACT INFECTIONS

Chlamydia trachomatis Infection: Diagnosis, Prevalence and Immunopathogenesis

Sexually transmitted infections (STIs) continue to represent a major public health concern worldwide, despite aggressive STI control efforts undertaken during the last several decades. A PCR has been standardized at the NIRRH to screen *C. trachomatis* infection and study the immunopathogens associated with this infection in 91 subjects enrolled and tested for RTI's/STI infections. The results revealed that the infection rate of *C. trachomatis* has decreased amongst women attending the gynecology clinic. Molecular HLA typing has been standardized to study the host factor. Of the infected pregnant women who were treated and followed up during the year, 4 had normal delivery and 1 had a cesarean section. All the babies were healthy and free of infection.

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

An HIV receptor/binding 160 kDa sperm protein on spermatozoa, that is bound specifically by gp120 HIV envelop glycoprotein as well as cell free HIV has been identified by Western blot analysis. To identify and isolate cDNA encoding the 160 kDa sperm protein, human testicular λ ZAP cDNA library is being immuno-screened using gp120 and its antibody. The positive clones will be further screened to isolate a full length cDNA clone for 160kDa sperm protein.

Reproductive Tract Infections: A Clinical and Microbiological Study in Women

Asymptomatic disease is responsible for frequent and severe long term morbidity of RTIs in women and for the persistence and spread of STIs in the community.

Study was initiated to evaluate the relationship between clinical manifestations and microbiological diagnosis of common RTIs (bacterial vaginosis, candidiasis, trichomoniasis and *C. trachomatis*) in women at low risk for RTIs and to assess the therapeutic response using clinical and microbiological diagnosis. A total of 560 women attending the NIRRH Family Welfare clinics have been screened and enrolled for participation in this study.

Eighty percent of the women came for their scheduled follow up and completed the study. Women (34) who were positive for *C. trachomatis* by DFA at the initial examination were given necessary treatment. Male partners were treated for *C. trachomatis* and *Trichomonas* infections identified in the women. Standard therapy was administered as defined in the WHO guidelines. Observations suggest that RTI infections, single or multiple, do occur in asymptomatic women and simple, easy methods for their detection need to be incorporated into the primary health care system. Also, the women and community need to be sensitized to distinguish between normal physiological, cyclical changes and abnormal symptoms which definitely need medical intervention. The other important aspect is the need to ensure compliance of therapy and recommend preventive measures to reduce transmission between partners.

MATERNAL HEALTH

Contraceptive Knowledge and Practices of Women Requesting Medical Termination of Pregnancy

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

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



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

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

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

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

Induced Abortion and Concurrent Adoption of Contraception in the Rural Areas of India

To obtain information from rural married women regarding their attitude, behaviour,

practices and utilization of services for induced abortion and concurrent acceptance of contraception, a total of 1851 women who had an induced abortion during the previous 3 years were interviewed in detail during a cross sectional survey carried out in 13 states. The main reason for seeking abortion was "don't need any more children" (42%) and "don't need any more daughters" (12.4%) (Fig. 17). Around 46% women accessed abortion services from private clinics as compared to government hospital (37%) and primary health centre/community health centre (PHC/CHC) (14.0%) (Fig. 18). The decision to terminate pregnancy and place of abortion was made by the husband in 42.8% and 52.5% respectively. Regret for abortion was expressed by 29.6% women. However, only 7.2% said they would not advise others for induced abortion. Nearly one half of the women undergoing abortion accepted a family planning method

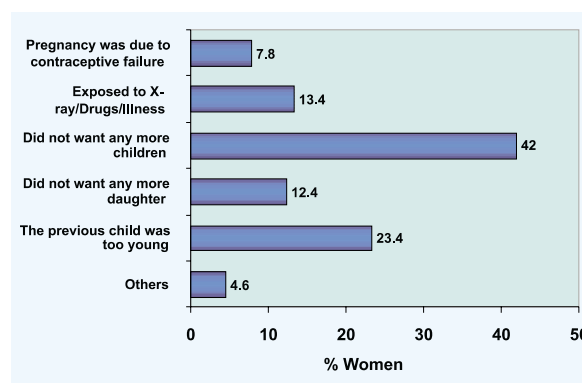


Fig. 17. Reasons for induced abortion.

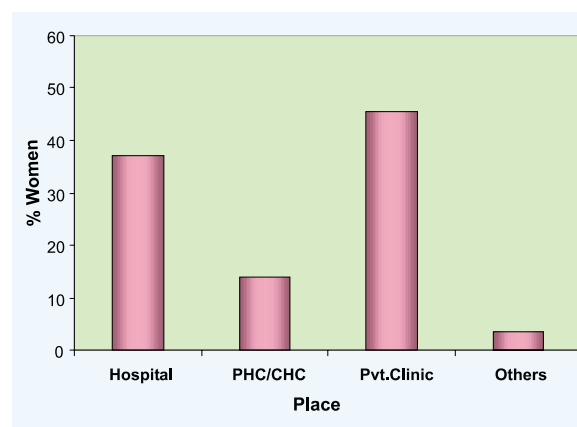


Fig. 18. Place of induced abortion.

concurrently; of these uterine device/oral contraceptives and a permanent method was adopted by 37.2% and 49.1% respectively. Acceptance of vasectomy by male partner was found to be low (1.3%). "Husband objected" (32.3%) was the main reason for not accepting post abortion contraception. Majority of the acceptors said they would recommend to others the same place where they had undergone abortion, thus indicating their satisfaction with the source and services received. There is need to strengthen and introduce safer methods of abortion services at PHC/CHC level to meet the demand in rural areas. In addition, counselling for post abortion contraceptives should be provided to the couple so that they can make an informed choice.

Feasibility of using Misoprostol at the Peripheral Level for Prevention of Post Partum Haemorrhage (PPH)

Active management of the third stage of labor, a known evidence based intervention that includes administration of a uterotonic, can prevent PPH due to uterine atony. Misoprostol (oral prostaglandin) has a tremendous advantage over other currently available uterotonics as it is inexpensive, stable at room temperature and is given orally. It has minor side effects that are self-limiting and usually do not require any medication.

A community-based task force study is ongoing at Pune, Cuttack, Jaipur, Baroda and Madurai to study the feasibility of administration of oral misoprostol for prevention of PPH by the paramedical worker at PHC level. this project would serve as a model applicable to rural settings throughout India and in other developing countries. The project is being carried out through ICMRs network of HRRCs and the DHO/CMO of the selected district. A total of 30 PHCs have been selected and randomly allocated as intervention or non intervention areas. In the intervention areas the active management of third stage of labor included administration of 600 mcg of misoprostol tablet after delivery of baby, early clamping and cutting of cord, and removal of placenta by controlled cord traction whereas in the non-intervention PHC the

existing practice for prevention of PPH under the RCH programme is being provided. Blood loss after delivery is being measured using a calibrated blood collection drape.

Interim analysis of 467 and 373 women who received and did not receive the intervention respectively indicated that they were similar in respect to age, parity, education and occupation. Majority (79%) of the women were in the active phase of labor, about 2/3rd of women in both the groups had haemoglobin less than 10.0 g%. The IIIrd stage of labor was managed by the peripheral health workers in the intervention areas according to the guideline in almost all the deliveries (99%). There was significant reduction in the duration of IIIrd stage of labor in the intervention group as compared to comparison group (Fig. 19).

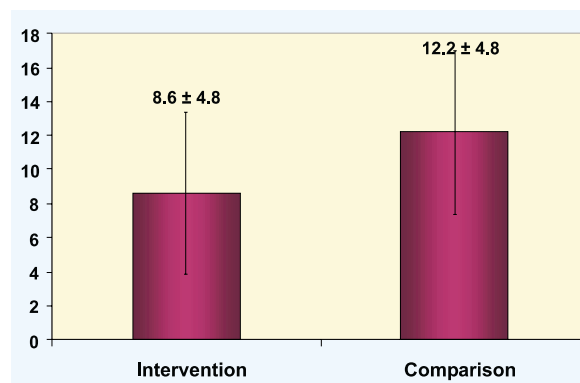


Fig. 19. Duration of third stage of labor.

The incidence of PPH was low (<1%) in both intervention and comparison areas. Only 0.9% cases in intervention area and 0.8% in comparison area had PPH (blood loss >500 ml) and one woman in intervention area was given blood transfusion at the medical college. However, average blood loss was significantly lower in intervention area compared to control area (Fig. 20).

Side effects like nausea and vomiting were comparable in both groups. An increased number of women had moderate to severe shivering in the intervention area. Similarly, rise in temperature >38° C was observed in intervention and comparison group in 4.5% and 1.3% women respectively. However, these side effects were self-limiting and did not require any treatment.



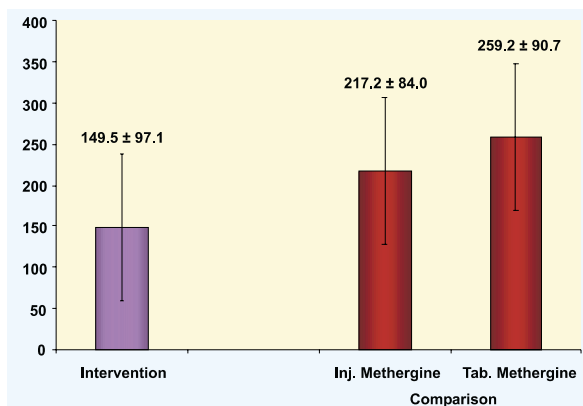


Fig. 20. Mean blood loss at 1 hr.

Management Practices of Meconium Stained Amniotic Fluid and Meconium Aspiration Syndrome

An exploratory study was carried out at 30 HRRCs with the objective to assess the management practices of obstetric cases with meconium stained amniotic fluid (MSAF) and to study morbidity and mortality in newborn babies with meconium aspiration syndrome (MAS), a major complication during delivery and childbirth. From among deliveries conducted at HRRCs during the study period, 7619 women with MSAF were enrolled in the study. Of these, 644 (8.5%) babies born to these mothers developed MAS. There was wide variation between centres at KMC, Chennai (2.7%) and medical college, Madurai (35.8%) reporting the lowest and the highest number of MAS babies respectively. The mean age of women with MSAF was 24.3 ± 4.0 and in 52.1% it was their first pregnancy. The character of the meconium was thick in 33.7% and 18.3% of these babies developed MAS. Forty six percent women in labor were managed by low segment cesarean section, whereas 8.6% were managed by vacuum extraction and forceps delivery. Forty five percent women who were in established labor delivered normally. Amnio infusion was done in around 14.9% of the MSAF cases. Babies born to mothers who were not given oxygen inhalation (71.0%) were at higher risk of developing MAS. No such association was found in respect to oxytocin drip amnio infusion, which were used for management of labor. The babies born to mothers with fetal distress (44%), non-progress

of labor (12.2%) and prolonged rupture of membrane during labor (4.3%) were at very high risk of developing MAS. Of total deliveries, 3.4% neonatal deaths, 0.5% still births and 0.3% intrapartum deaths amounting to 4.2% deaths were reported. In 91.5% babies who did not develop MAS, there were 0.3% intrapartum deaths, 0.6% still births and 1.6% deaths during neonatal period. Out of 8.5% MAS babies, 23.1% died during neonatal period and 0.3% were intrapartum deaths. The main cause of neonatal deaths was birth asphyxia accounting for 53.9% of the total 4.2% deaths of newborns in the study.

Diagnostic Markers for Osteoporosis

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

Biochemical markers of bone turnover were estimated in serum samples from 206 women in the age group 20-60 yr. The preliminary data indicate a distinct change in the biochemical markers of bone turnover, reflecting the age related changes in bone metabolism. The level of markers in 60 menopausal women who had hormone replacement therapy showed a significant drop (9-12%) within 3 months of therapy.

A study has been initiated to develop an in-house ELISA for osteocalcin, extracted and isolated from bovine bones. Antisera have been raised.

For studying mechanism of action of accelerated bone resorption during menopause, immunoassays have been standardized for interleukin 1, interleukin 6 and tumour necrosis factor alpha.

CHILD HEALTH

Home Based Management of Young Infants

Current neonatal mortality rate in India is 45/1000 live births accounting for almost two thirds of the infant deaths. In view of developing a model for reduction of neonatal mortality a project on home based management of newborn is being carried out in rural areas of five states in the country as more than 60% deliveries take



place at home setting in rural India. There are two models of intervention—in one arm a community based worker chosen by the community and in the other an Anganwadi Worker (AWW) at the ground level are the provider of intervention. The proposed package of home based intervention comprises care of the infant at birth, care of normal and low birth weight babies, treatment of local infections, referral of sick infants, health education, provider partnership and community participation. The workers (SR/AWW) under the project receive extensive training spread over a period of 6-9 months. A careful post training evaluation is done to judge necessary skills transfer. The impact of intervention is to be assessed by doing baseline and endline survey.

Baseline survey included mortality survey, facility survey and survey of practitioners. Major activities of formative phase included ethical clearance, interphasing with health system, baseline survey, randomization and training/skill building of SRs/AWWs for providing services and training of ANM/LHVs who supervise the activities of SRs/AWWs.

Baseline survey was carried out in 9 PHCs from which 6 PHCs similar in terms of neonatal mortality rate were finally randomized into different intervention arms. The results of the mortality survey reveal that neonatal mortality rate was 48.5, 45.8, 41.1, 77.9 and 48.3 per 1000 livebirths and infant mortality rate 90.9, 62.2, 56.7, 113.9 and 59.8 per 1000 live births in Lucknow (U.P.), Cuttack (Orissa), Patna (Bihar), Udaipur (Rajasthan) and Wardha (Maharashtra) respectively. Fig. 21 shows the percent neonatal deaths in first four weeks of life in these areas.

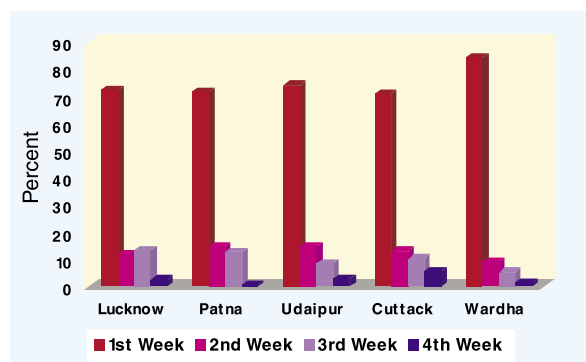


Fig. 21. Percent neonatal deaths in first four weeks of life.

Training of master trainers and SRs/ AWWs in step ladder fashion *i.e.* class room training followed by field practice is ongoing. Four master trainers from each centre have been trained at Gadchiroli. These master trainers are providing training to SRs at the respective sites.

National Neonatal Perinatal Database

A fundamental prerequisite for planning, implementation and monitoring the health care programmes is to ensure an accurate ongoing assessment of morbidity and mortality derived from a reliable database. National neonatal-perinatal database network established at the tertiary hospitals collects data on neonatal-perinatal morbidity and mortality on a standardized protocol developed for this purpose. There is paucity of information on morbidity and mortality of neonates admitted at district/subdistrict hospitals. Eight HRRCs (KEM Hospital, Mumbai, K.G. Medical College, Lucknow, JIPMER, Pondicherry, Baroda Medical College, Baroda, PGIMER, Chandigarh, Kasturba Hospital, Delhi, AIIMS, N. Delhi and KEM Hospital, Pune) collected information on intramural deliveries, extramural births and maternal parameters from the district hospitals on structured format.

Data received on 18,074 neonates have been analysed. Three quarter of women who delivered at district hospitals had at least 3 antenatal contacts. Around 80% were normal deliveries and 16% were delivered by LSCS. Mean birth weight was 2741 ± 660 gm, 22.5% were low birth weight while 7.2% were preterm. Around 96% babies cried after birth within 1 min, 2.2% between 1-5 min and 1% after 5 min. Resuscitation at birth was required in the form of oxygen in 4.4%, bag and mask ventilation in 2.1%, chest compression in 0.6% and medications in 2.1% babies delivered.

Common morbidities in these neonates included septicemia (0.7%), hyperbilirubinemia (1%) hypothermia (0.8%), hypoglycemia (0.8%), seizures (0.4%) and apnoea (0.3%), antibiotics (6.5%), oxygen (4.5%), blood/plasma transfusion 0.1% and phototherapy (0.5%). There were 1.0% neonatal deaths, while 0.3% left the hospital against medical advice and 1.9% were referred



to higher centres. Single most important cause of death included perinatal asphyxia (45%), prematurity (30%), infections (3.9%), malformations (2.3%) and others (9.4%) and cause was not established in 10.3% of cases. (Fig. 22). Age at death is shown in Fig. 23.

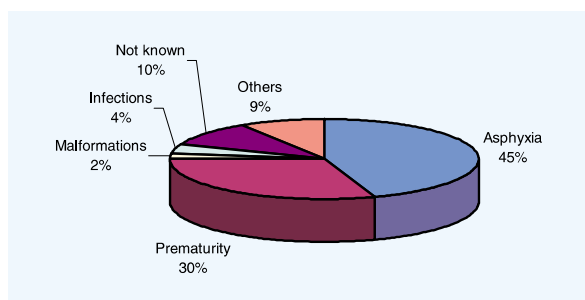


Fig. 22. Primary causes of death (inborn babies)

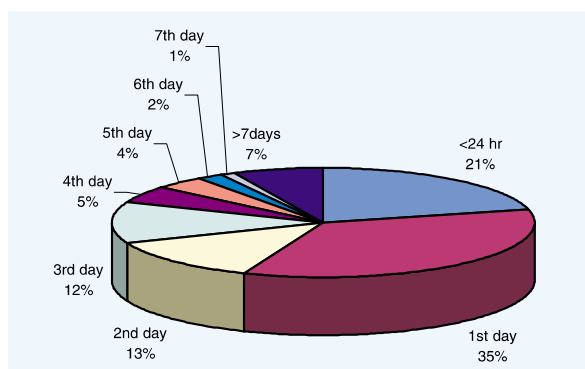


Fig. 23. Age at death

Morbidity and Mortality Details of Outborn Babies

A total of six participating ICMR HRRCs collected data for a cumulative 42 months on 681 outborn babies. Fifty six percent babies were born in hospitals while 29% were born in home settings; 45% babies were low birth weight while 30% were preterm. At admission the mean weight of babies was 2323 gm (± 618) and mean age was 5.4 (± 7.0) days. The morbidities included respiratory distress (38.7%), septicaemia (25.6%), hyperbilirubinaemia (9.8%), seizures (4.5%) and hypothermia (4.5%). Overall mortality was 16.7% while 3.5% babies left the hospital against medical advice and 9.5% were referred to higher centres. Mean age at death was 1.1 (± 4.1) days. Single most important cause of death included perinatal asphyxia (50.2%), sepsis (36.1%),

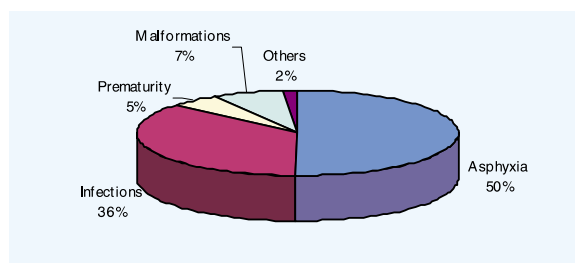


Fig. 24. Primary causes of death (outborn babies)

prematurity (5.2%), and malformations (7.0%). (Fig. 24).

Cognitive Problems in Low Birth Weight Children

Due to paucity of Indian data an in-depth longitudinal study of cognitive and learning problems in low birth weight neonates (followed upto the age of 12 yr.) was carried out at KEM Hospital, Pune to assess their potential for learning (intelligence quotients) and actual achievements in learning.

A total of 180 children (73 girls, 107 boys) with birth weight <2000 gm were prospectively followed up since birth for a period of 12 yr. Full term children comprised 81.2% while rest were preterm. Out of 147 preterm children, half were appropriate for gestational age (AGA) and the other half were small for gestational age (SGA). Amongst all the SGA children 31% were full term and 69% preterm.

Low birth weight, especially the very low birth weight (VLBW) (birth weight <1500gm) and the preterm small for gestational age children have lower IQs compared to normal controls (Fig. 25). Writing and mathematical

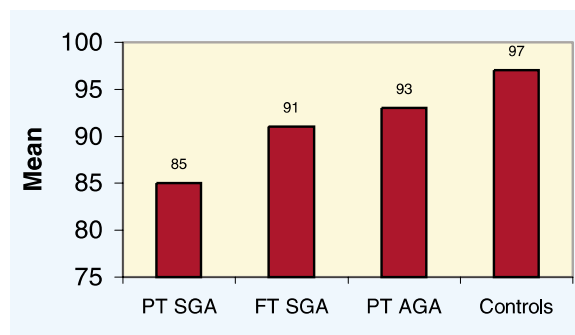


Fig. 25. Mean IQ at 12 yr.

skills of VLBW and preterm children with gestation less than 32 wks were particularly poor. All children from the study group performed poorly on the ABC Movement test. Girls performed poorly compared to boys in body balance and ball skills, but were somewhat better in manual dexterity. The academic performance of the low birth weight children was also poor. There were more failures with poor academic performance. The IQs correlated well with marks in the examination. Environmental factors like socio-economic status and spaciousness of the house and parental education greatly influenced the IQ. The only biological risk factor influencing IQ at 12 yr. was the birth weight.

ADOLESCENT REPRODUCTIVE HEALTH

Knowledge, Attitude and Felt Need of Emergency Contraception amongst Adolescents and Youth. Health Care Providers and Family Planning Counsellors in and around Mumbai

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

The overall awareness among adolescents and youth regarding EC was 4.2%, parents - 8% teachers - 12.2% and service providers - 48% (i.e., 75% among Obstetricians and Gynecologists, 46.7% among other medical practitioners, 40% among family planning counsellors and 31% among nurses). Among WHAs and NGOs, 18.5% reported being aware of ECs. The awareness

among chemists and drug store owners was 60% (68% among medical and fancy store personnel and 40% among medical stores personnel). However, even among those who were aware of EC, most of them did not have correct knowledge.

To prevent unwanted pregnancies, majority of the WHAs and NGOs opined that it was necessary to promote safer sex. Majority (37%) of WHAs and NGOs and more than one-third of the parents felt that EC methods should be provided through doctor's prescription. About 90% parents were of the opinion that sex education should be provided to prevent unwanted pregnancies. Six of every 10 parents and more than half of WHAs and NGOs (55.6%) and chemists feared that EC may be misused and may affect women's health adversely.

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

About 71% of adolescents and youth, two-thirds of service providers, 70.9% teachers, 50.3% parents, and 88.9% of WHAs and NGOs opined that EC should be included in the National Family Welfare Programme. Seven in 10 teachers and 71.7% of parents viewed that easy accessibility and availability of EC would increase the use among married and unmarried people.

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

The study indicated that awareness of EC among various groups is low, attitude towards



EC is positive and felt need of contraception is observed among various groups. All the respondents expressed their desire to know more about contraception and EC. The study underscores the pressing need to increase awareness and knowledge of EC through effective intervention programmes such as audiovisuals (television and radio), print media (posters, pamphlets, brochures and flipcharts), help-line, hotline and internet services, so that unwanted pregnancies could be prevented.

Improving Reproductive Health of Adolescents: An Urban School Based Approach

A study was launched in two schools and two colleges to assess the knowledge, attitude and practices about reproductive health among 300 adolescents aged 11-19 yr from different socio-economic groups and to operationalise Adolescent Friendly Centres in those settings and to assess the uptake of these services.

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

Knowledge on various aspects of reproductive health including HIV/AIDS was greater among boys than girls. Adolescents from the lower socio-economic class reported more reproductive health problems as compared to those from middle socio-economic class. Problems reported by girls were mainly dysmenorrhoea, irregular bleeding, excessive vaginal discharge with foul smell and pruritis vulvae (Fig. 26). Boys reported itching of genitals, painful micturition and discharge from urethra (Fig. 27).

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

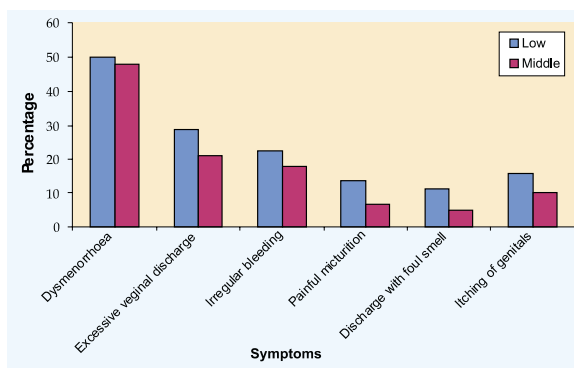


Fig. 26. Reproductive health problems of girls aged 11-14 yr.

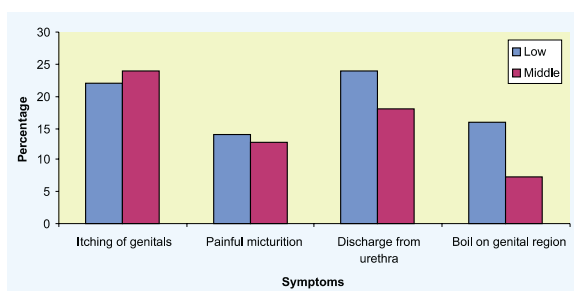


Fig. 27. Reproductive health problems of boys aged 11-14 yr.

quacks. Adolescents from the middle socio-economic class went mainly to private practitioners.

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

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

Reproductive health check up and counselling was carried out for boys and their



parents. Preliminary analysis and experiences from these studies suggest that adolescents have a quest for information on reproductive and sexual health issues. They are willing to approach adolescent friendly centres for various reproductive health problems, however, their health seeking behaviour is poor. Many of the reproductive health problems like anaemia, genital anomalies remain undiagnosed due to

ignorance on the part of adolescents themselves and their parents. School health checkups which are part of our system lack reproductive health focus which needs to be incorporated in order to reduce the disease burden of adolescents in adult life and hence a comprehensive approach with medical/health check up is very essential in addition to providing IEC.

