

Fig. 17: Quantitation by image analysis software. In both (A) testis and (B) epididymis 80kDa HSA expression shows androgen regulated expression pattern.

#### 1.4 Modulation of c-kit Proto-Oncogene Function During Spermatogenesis in Mice (*Funded by Indo-US Program*)

Principal Investigator: K.V.R. Reddy

Project Associates: A.P. Sikarwar and Clara Aranha

Duration: 2003-2008

## Role of c-kit in germ cell proliferation and migration *in vivo*

The rising incidence of male infertility is a cause of great concern. Approaches to this problem will need a basic understanding of the process of spermatogenesis at the molecular level. In our earlier studies, it has been shown that, c-kit/SCF plays a critical role in the differentiation of germ cells and their survival *in vitro* (Annual Report 2003, p 48). During this year, studies were extended to determine *in vivo* the role of c-kit primordial germ cells (PGCs) in PGCs formation, proliferation and migration. It is known that, PGCs are the earliest detectable cells in the extra embryonic mesoderm at 7.0 days post coitum (dpc). At 8.0 dpc, normally these cells leave the base of allantois and migrate into the hindgut epithelium. Subsequently, at 9.5 dpc they migrate along the hindgut and by 10.5 dpc they reach the dorsal mesentery and finally by 11.5 dpc they enter the newly formed genital ridge.

For the study, pregnant CD1 mice were injected intradermally with 2 $\mu$ g of c-kit blocking antibody on 9.5 dpc and 11.5 dpc. Twenty four hours later, these animals were administered with Brdu (25 $\mu$ g/mice) subcutaneously. The mice were sacrificed after 2 hrs and embryos were collected. The effect of c-kit antibody on the formation and migration of PGCs was evaluated by immunohistochemistry. The embryos were fixed (methanol: acetic acid, 3:1), rehydrated and stained with freshly made alkalinephosphatase (ALP) stain. The number of Brdu positive PGCs on 12.5 dpc were counted in hindgut, dorsal mesentery and genital ridge regions (Fig. 18). The incorporated Brdu was detected using anti Brdu and FITC labeled streptavidin antibodies. The number of PGCs positive for ALP and Brdu were drastically reduced after c-kit antibody treatment, suggesting c-kit plays a critical role in the formation, proliferation and migration of PGCs.

## Expression of c-kit and SCF genes in the testes of mouse during postnatal development

The c-kit protooncogene (encodes for a transmembrane receptor), and its ligand Stem Cell Factor are associated with maturation and differentiation of germ cells. Loss or alterations in the expression of either of these genes lead to arrest of spermatogenesis. In the present study, we examined the stage-specific expression of c-kit and SCF during postnatal development in the testes of mice. Total RNA was extracted from the testes of mice on days 5,10,20,40 and 60. Gene specific primers for c-kit (5'- ACC CAC AGG TGT CCA ATT ATT C -3' F), (5'- TGG CGT TCA TAA TTG AAG TCA C -3' R) and SCF (5'-CTC CCT TAG GAA TGA CAG CA-3' F), (5'-AAG CAC TCT GCT CCA ACA AC-3' R) were designed to amplify the respective nucleotide fragments by PCR. The expected product sizes of 403bp and 429bp were

obtained for c-kit and SCF respectively. Results revealed that both genes were expressed differentially during testicular development, despite similar concentrations of template cDNA used, compared with Cyclophilin-A controls showing similar signal intensity. Maximum signals were observed on days 10, 20 and 40 for c-kit gene and 40 and 60 days for SCF gene, suggesting that upregulation of expression of these gene could be due to the transition of spermatogonia to spermatocytes and spermatids (Fig. 19). PCR generated products were quantitated by densitometry (Fig. 20).

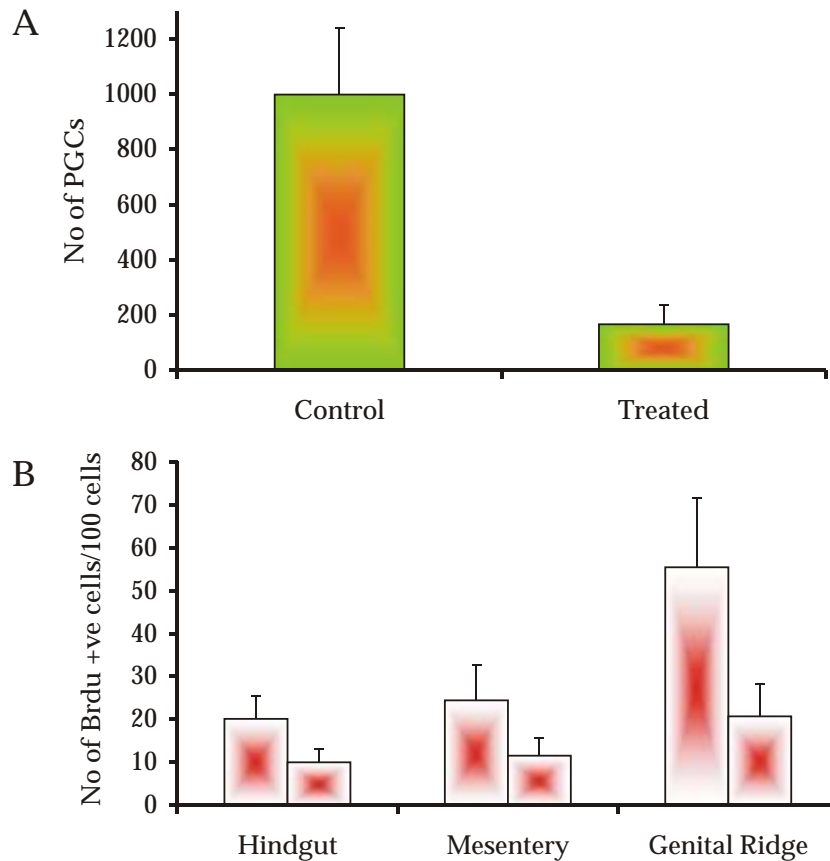


Fig. 18: PGCs +ve for (A): AIP and (B): Brdu, at 12.5 dpc embryos.

### c-kit gene silencing *in vitro* using RNAi approach

The application of RNAi interference in mammalian cells has revolutionized the field of functional genomics. RNAi is a highly sequence specific mechanism of post transcriptional gene silencing, mediated by double stranded interfering RNA (dsRNA). The size of the siRNA ranges from 19 to 24 oligonucleotides (nt) which can be used to target any gene of interest.

Chemically synthesized, target specific 21 nt siRNA (sense and antisense) with 2-nt 3' overhangs of identical sequence were commercially procured as deprotected duplexes (Qiagen). The SiRNA sequence used for targeting c-kit mRNA was from the position 1240-1261 (c-kit accession numbers NM\_021099 and  $\beta$ -actin accession numbers NM\_0011101) (Fig. 21). The siRNA was tested in c-kit expressing P815 mouse mastocytoma cell lines for determining its ability to silence the endogenous c-kit gene expression.

### Cell culture and transfection

In the present study, we report for the first time that, 21 nt siRNA duplexes induced c-kit gene knockdown in c-kit expressing P815 cells. Briefly, the cells were grown in DMEM containing 10 percent FCS at 37C in a humidified atmosphere with 5 percent CO<sub>2</sub> and maintained exponential growth. The day before transfection, trypsinized cells were cultured in 24-well plate at a density of 2X10<sup>5</sup> cells/well in a volume of 500 $\mu$ l medium.

Lipid-RNA complexes were prepared by incubating 0.15 $\mu$ l (5 nM) of appropriate siRNA with 3 $\mu$ l HiPerFect transfection reagent at RT for 10 min. The plated cells were incubated with transfection mixture at 37C for 6 hrs after which, normal growth medium was added. Further incubation was carried out for 72 hrs to achieve > 80 percent down-regulation of c-kit gene expression. The experiment was repeated and specificity of gene silencing was confirmed.

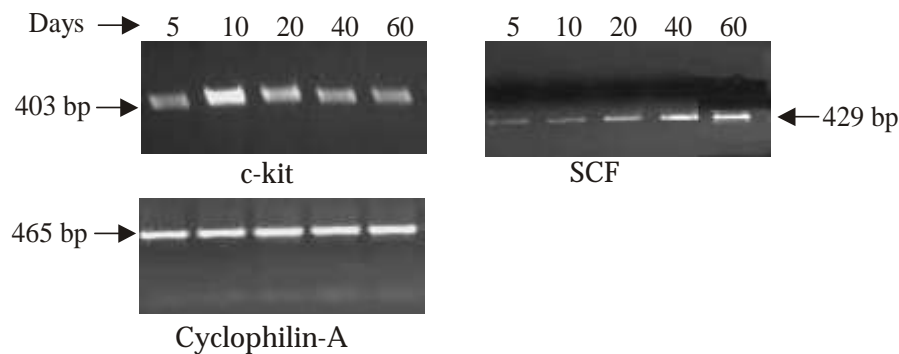


Fig. 19: PCR analysis of c-kit and SCF genes in the testes of mice during different stages of development.

### Immunofluorescence analysis

Cells expressing c-kit were transfected with various concentrations of siRNA duplexes (1.87 - 75 ng) targeted against c-kit. The transfection efficiency, siRNA effects and cellular uptake were analyzed in living cells by fluorescence microscopy. For comparison, siRNAs of  $\beta$ -actin and Cy3 were used as positive and negative control respectively. It was observed that, c-kit siRNA treated cells showed

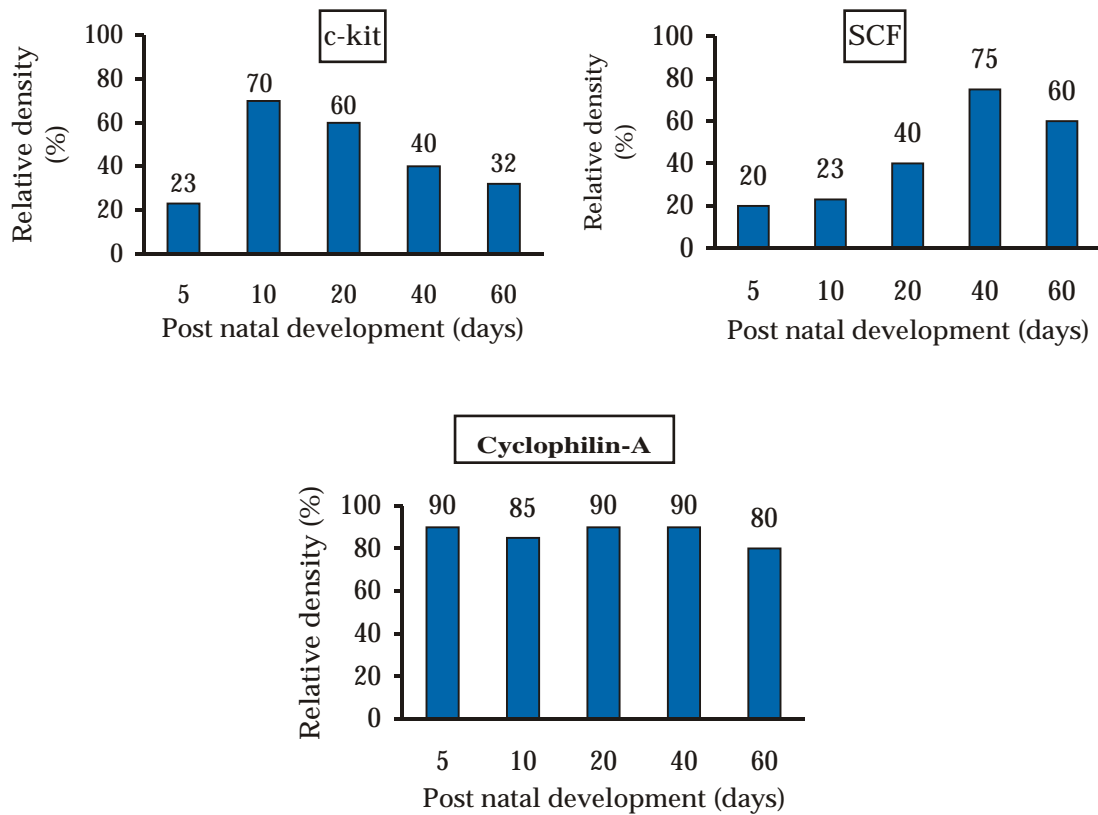


Fig. 20: Quantitation of gene signals by densitometry. Control used Cyclophilin-A.

A	<p><b>5' - TTC CGT GAC ATT CAA CGT TTA - 3'</b> (Target sequence)            5' - CCG UGA CAU UCA AGC UUU A dT dT 3' (sense)            3' - dT dT UAA ACG UUG AAU GUC ACG G dA dA 5'(antisense)</p>
B	<p><b>5' - AAT GAA GAT CAA GAT CAT TGC - 3'</b> (Target sequence)            5' - UGA AGA UCA AGA UCA UUG CdT dT 3' (sence)            3' - GCA AUG AUC UUG AUC UUC AdT dT 5'(antisense)</p>
C	<p><b>5' - AAT TCT CCG AAC GTG TCA CGT - 3'</b> (Target sequence)            5' - UUC UCC GAA CGU GUC ACG UdT dT 3' (sense)            3' - ACG UGA CAC GUU CGG AGA AdT dT 5'(antisense)</p>

Fig. 21: Target mRNA, sense and antisense sequences for (A): c-kit, (B): -actin, and (C) Cy3.

significant reduction of c-kit expression at 72 hrs post transfection. Similar reduction was seen in  $\alpha$ -actin siRNA treated cells. However in Cy3 siRNA treated cells no such reduction was noted (Fig. 22). The number of cells displaying reduction in c-kit silencing were quantitated and normalized to 100 percent (Fig. 23). Further, it was also observed that, c-kit siRNA transfected cells showed arrest of growth followed by apoptosis, suggesting the critical role played by c-kit in maintaining growth, differentiation and survival of cells.

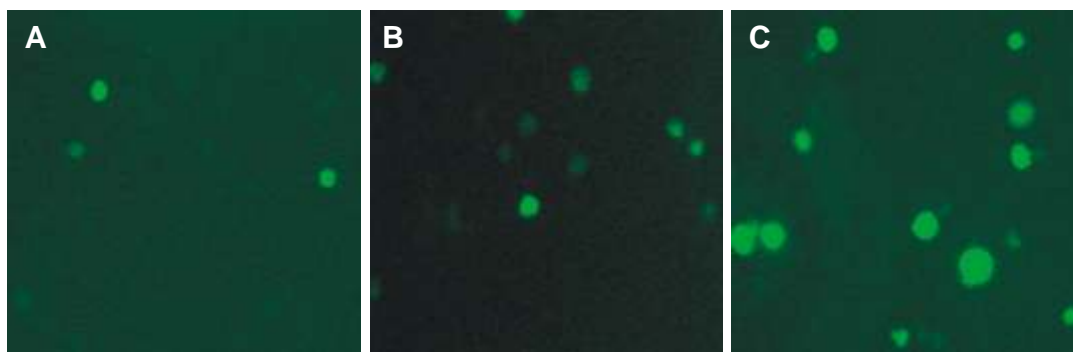


Fig. 22: Immunofluorescence analysis of gene silencing by siRNA duplexes (37.5ng) in P815 cells (A=c-kit, B=  $\alpha$ -actin and C=Cy3) (Magnification x 40).

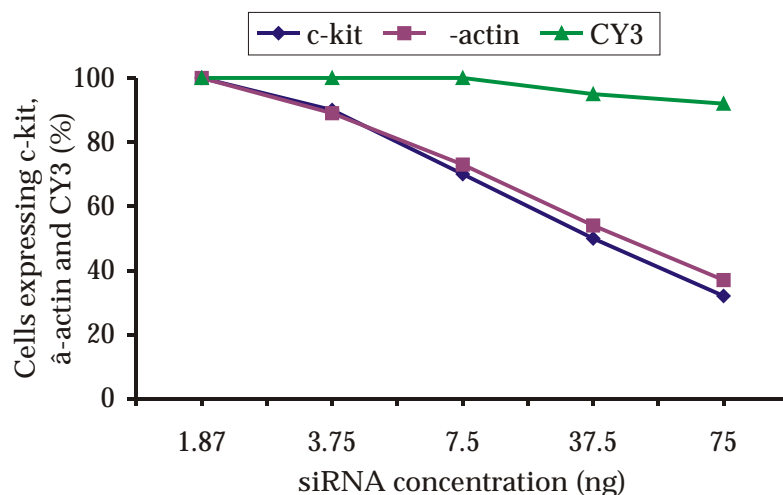


Fig. 23: C-kit expressing P815 cells were transfected with various concentrations of siRNA duplexes (1.87-75 ng) targeted against c-kit,  $\alpha$ -actin and Cy3. siRNAs of  $\alpha$ -actin and CY3 were used as positive and negative controls, respectively.

#### Detection of protein knockdown by immunoblot analysis

ELISA and immunoblot analysis were used to confirm the levels of c-kit protein in siRNA transfected P815 cells. After 72 hrs post transfection, the cells grown in 24-well plates were washed in ice cold PBS and harvested. Harvested cells were lysed with lysis buffer and used for ELISA, SDS PAGE and Western Blot. Both

ELISA and immunoblot (Fig. 24) results revealed significant decrease in c-kit levels in transfected cells and these observations were consistent with the immunofluorescence data.

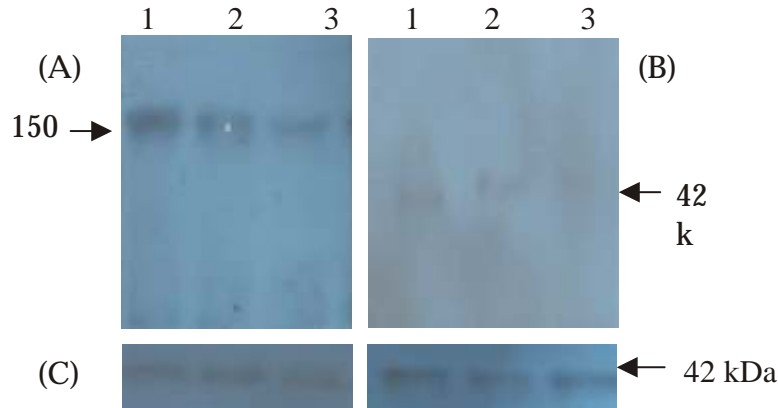


Fig. 24: Immunoblot analysis of gene specific siRNAs. The untransfected (Lane 1) and transfected cells with 5nM (Lane 2) and 10nM (Lane 3) siRNA, demonstrate that the levels of c-kit (A) and  $\beta$ -actin (B) protein were reduced in transfected cells. For comparison  $\beta$ -actin (C) was used as loading control.

#### Off target effect of RNAi in silencing the c-kit gene expression

It is known that transfection of siRNA can lead to off target effects where siRNA affects the expression of nonhomologous or partially homologous gene target, which may give misleading results. The present experiment was carried out to determine the effect of c-kit siRNA on the expression of other genes. This was confirmed by selecting one of the genes, Oct-4 (known to be involved in the differentiation of germ cells). Immunofluorescence and Western Blot analysis revealed that, c-kit siRNA duplex does not inhibit the levels of Oct-4 protein, suggesting that c-kit gene silencing is specific.

#### Northern blot analysis

Northern blot hybridization was carried out in order to prove that the observed siRNA knockdown phenotypes were due to degradation of target c-kit mRNA in the gene silencing (c-kit &  $\beta$ -actin) and expressing cells (Cy3). Total RNA was extracted from these cells at 72 hrs post transfection and fractionated on a denaturing formaldehyde agarose gel and transferred to a nylon membrane. The c-kit message was probed using a 602 bp nucleotide sequence (consisting of target mRNA sequence). Gene specific primers (5'-CTC GGC AAG AGT TGA CGA TTC T-3' F), (5'-CTA TGG AAC TCT GAA CCA CCA G-3' R) were designed to amplify a 602 bp PCR product. The PCR product was digoxigenin (DIG) labeled and blots were hybridized O/N with labeled probe. The results indicated a significant reduction in c-

kit and  $\beta$ -actin mRNA signals in transfected cells (Fig. 25). With this approach, we observed a highly efficient siRNA specific for c-kit &  $\beta$ -actin which shows potent knockdown (~80%) of these genes c-kit at 37.5 ng concentration of siRNA. Further studies are in progress.

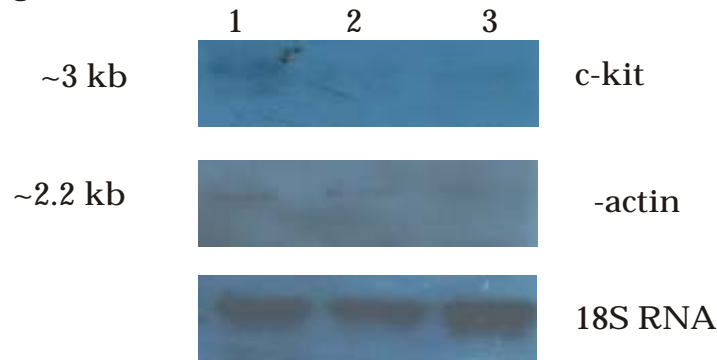


Fig. 25: Characterization of c-kit specific siRNA. The untransfected (Lane 1) and transfected cells with 5nM (Lane 2) and 10nM (Lane 3) siRNA demonstrating the specific degradation of c-kit &  $\beta$ -actin mRNAs. As a control for loading equal amount of mRNA in each lane, 18S ribosomal RNA probe was used.

### 1.5 Studies with FSH Binding Inhibitor: Functional Significance of FSH Modulators from Follicular Fluid in Ovarian Pathophysiology (*Partly funded by CONRAD*)

Principal Investigator: Tarala D. Nandedkar  
 Project Associates: Swati Kulkarni, Smita Mahale,  
 Rajshree Navlakhe, S. Rewdekar,  
 S. Ghanekar, P. More  
 Duration: 2003-2007

Gonadotropins - FSH and LH play an important role in proliferation and development of ovarian follicles. However, in each ovarian cycle, out of the pool of follicles selected, only one/few ovulates. Therefore, it is well established that autocrine/ paracrine factors secreted by the ovary, directly or indirectly regulate gonadotropin action. Follicle Stimulating Hormone Binding Inhibitor (FSHBI) is one such factor purified by our group from human ovarian follicular fluid. FSHBI has a molecular weight of ~ 4 kDa. It has been shown to inhibit FSH binding to granulosa cells *in vitro*; while *in vivo* it induced atresia in mice and interfered with ovulation in bonnet monkeys. Partial N terminal 8 amino acid sequence of FSHBI has been deduced which is referred to as Octapeptide (OP). The synthetic analogue of FSHBI; OP demonstrated similar biological activity as that of native FSHBI in mice.

## Effect of OP in cycling bonnet monkeys

To study the effect of OP on the hormonal profile, normal menstruating bonnet monkeys were used as an animal model. The cyclicity of the animals was checked by taking vaginal swabs. The animals were divided into two groups control (n = 6) and treated (n = 6) (Fig. 26A and B). The treatment group was administered 250µg/day /kg dose of OP for 10 days, 3 - 12 post menstruation. The 5 animals from the treated group exhibited amenorrhea in the post treatment cycle. The controls were given 0.5 ml saline/day for 10 days, 3 - 12 post menstruation as shown in Table 2 and 3. These animals continued normal cyclicity (as confirmed by vaginal swabs). The circulating steroid levels of the treated and the control groups were estimated by ELISA.

Table 2: Duration of menstrual cycles in control group in pre-treatment, treatment and post-treatment cycles.

Animal No. (Control)	Duration of cycle (days) in <i>Macaca radiata</i>		
	Pre-treatment	Treatment	Post-treatment
C1	45	29	44
C2	29	28	28
C3	29	34	32
C4	29	30	27
C5	29	32	34
C6	28	31	27
	31.5 ± 2.69	30.67 ± 0.89	32.0 ± 2.67

The P<sub>4</sub> levels in the control group monitored for 3 menstrual cycles showed a cyclic pattern with levels remaining low (< 1ng/ml) in follicular phase and an increase in its levels in luteal phase. All 5 animals from the treated group exhibited amenorrhea for 73 ± 6.66 days. In the prolonged luteal phase, increase in P<sub>4</sub> levels was observed but the levels remained low. The P<sub>4</sub> levels in the 5 treated animals were 64 percent lower as compared to the respective pre-treatment cycles. The levels did drop down as expected follicular phase however, there was no withdrawal bleeding observed with such low level of P<sub>4</sub>. Thus, the peptide possibly has a drastic effect on P<sub>4</sub> levels and the study will be continued using a lower dose of OP.

The amenorrhea observed in OP treated group was similar to that reported by us earlier with the partially purified (hGF<sub>2</sub>) treatment in bonnet monkeys.

Table 3: Duration of menstrual cycles in pre-treatment, treatment and post-treatment cycles in treated group.

Animal No. (Treated)	Duration of cycle (days) in <i>Macaca radiata</i>		
	Pre-treatment	Treatment	Post-treatment
T1	27	61	30
T2	28	57	32
T3	28	90	28
T4	26	65	34
T5	32	92	36
	$28.2 \pm 1.02$	$73.0 \pm 6.66$	$32.0 \pm 1.29$

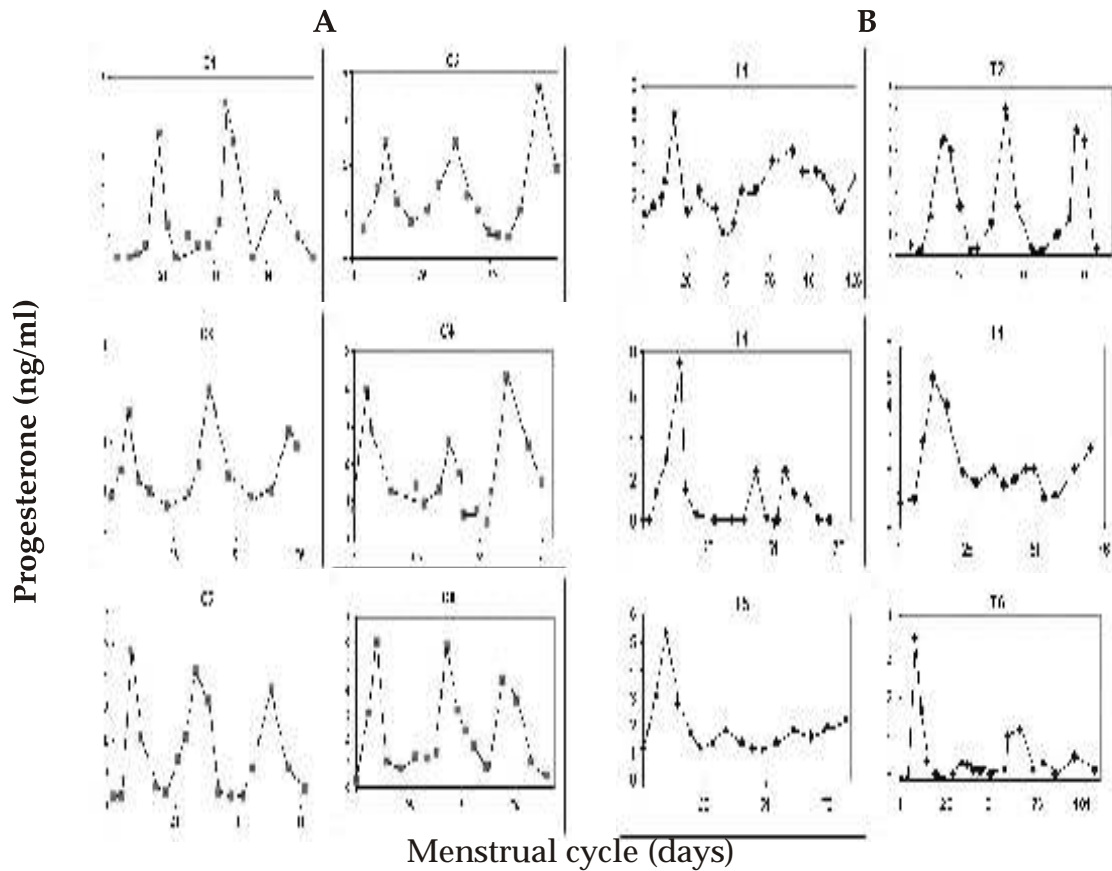


Fig. 26: (A): Control animals (n=6) 0.5 ml saline/day; (B): OP treated animals (n=5) 250 µg/day/kg body weight.

## 1.6 Factors Regulating early Folliculogenesis in Mouse Ovary (*Funded by Department of Biotechnology*)

Principal Investigator: Tarala D. Nandedkar

Project Associates: Shalmali Dharma, D.N. Modi

Duration: 2003-2007

In the mammalian ovary, early follicular development is gonadotropin independent. Interaction between the oocyte and granulosa cells possibly plays an important role in transition of primordial follicle to preantral stage. However, the molecular and cellular control of early follicular development and cell-cell interaction is complex and poorly understood. In the present study, we are aiming to identify the molecular pathways of early folliculogenesis by using a neonatal mouse model. For this study, we have screened the expression of 1200 genes in primordial, primary and preantral follicles by cDNA arrays in Day 2, Day 4, Day 6 neonatal mouse ovaries that contain the various developmental stages of these follicles, respectively. The results revealed that 26 percent genes were differentially expressed in Day 4 ovaries containing primary follicles as compared to Day 2 ovaries consisting of primordial follicles. Further additional 6 percent genes were detected in Day 6 ovaries containing preantral (secondary) follicles. The differentially expressed genes belonged to the number of functional categories such as growth factors, metabolism, cell cycle, transcription and translation, neurotransmitters. The differentially expressed genes like Anti-Mullerian Hormone (AMH), c-kit, Stem Cell Factor (SCF) are validated by using immunolocalization of proteins. Immunohistochemical staining of AMH was concentrated in the cytoplasm of granulosa cells of primary (Fig. 27b) and preantral follicles (Fig. 27c) but not in the primordial follicles at Day 2 (Fig 27a). The weak expression of c-kit was observed in the oocytes of primordial follicles (Fig. 27f) and gradually started increasing further in primary (Fig. 27g) and preantral follicles (Fig. 27h). Also, Stem Cell Factor was localized in the granulosa cells of primary (Fig. 27l) and preantral follicles (Fig. 27m) but not in the primordial follicles (Fig. 27k).

These results infer that primordial follicles are quiescent while major activities of cell-cell communication, production of local paracrine factors, are initiated in primary and preantral follicles of the mouse ovary. These observations may contribute to the elucidation of molecular and cellular pathways involved in follicle transition.

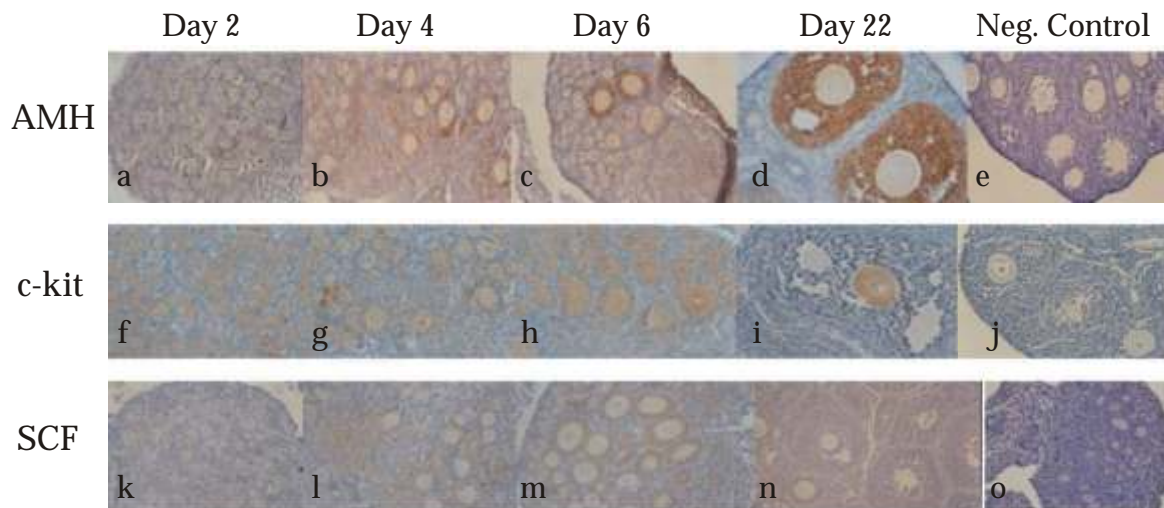


Fig. 27: Immunostaining for Anti-mullerian hormone (AMH), c-kit, Stem Cell Factor (SCF) during follicular development in the neonatal mouse ovary. Counterstained with haematoxylin (a, k) Day 2 ovaries; negative for AMH and SCF respectively. Intense AMH and SCF staining in the granulosa cells of (b, l) in the primary follicles on Day 4 and (c, m) in preantral follicles on Day 6 respectively. Intensity of c-kit immunostaining in the oocytes of primordial follicles (f), primary (g) and preantral (h) follicles. Day 22 (d, i, n) ovary used as a positive control for respective antibodies. On Day 22 (e, j, o) ovary, omission of primary antibodies yielded no staining represent the validation of immunohistochemical technique.

### 1.7 Acceptability and Continuation Rates of 2 Monthly Injectable Contraceptive: Norethisterone Enanthate (*Funded by Ministry of Health and Family Welfare*)

Principal Investigator: Shanta Chitlange

Project Associates: Kamal Hazari, D. Balaiah, Rashmi Shah, Lalita Savardekar, Neha Minde, Chitra Thosar, Shalini Baji, Pratibha Kokate, Dipika Belekar, P. Tapase, A. Khanvilkar.

Investigators and Participating Centres: Prof. H. Saini, Baroda  
 Prof. C. Alexander, Chennai  
 Prof. J. Mallick, Cuttack  
 Prof. A. Bhargava, Jaipur  
 Prof. T. Ghosh, Kolkata  
 Prof. V. Salvi, Mumbai, KEM Hospital  
 Prof. C. Doifode, Nagpur  
 Prof. S. Salhan, New Delhi

Duration: 2001-2007

During the reporting period, a two year follow up of women on injection Norethisterone Enanthate (Net-En) has been completed after enrolling 1209 women. Observations are based on 17268 months of use of Net-En. The number of women who had received between 1 and 12 injections during the 2-year period is shown in Fig. 28.

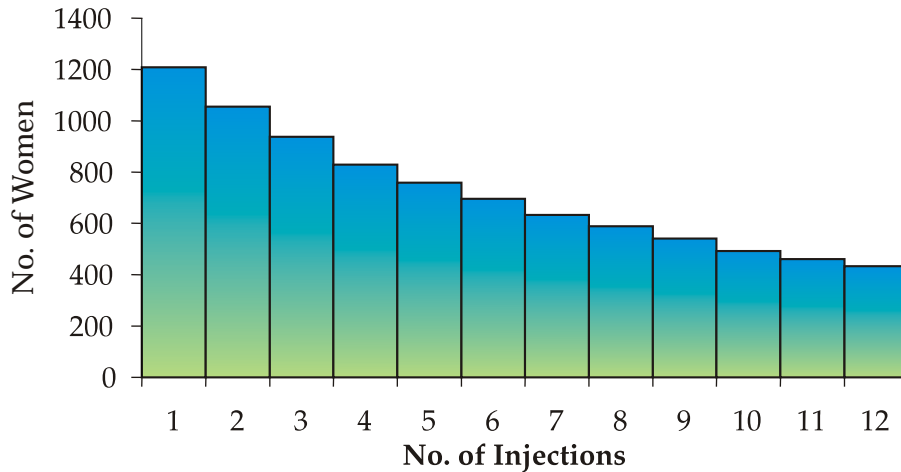


Fig. 28: Women who had received between 1 & 12 injections during the 2 years.

Of the 1209 women, 519 (42.9%) received first injection during menstruation, 438 (36.2%) received 6 weeks after delivery and remaining 252 (20.8%) after surgical abortion. While 721 (59.6%) women used some methods of contraception prior to entering the study (Table 4) the remaining 488 women did not use any contraceptive. The reasons for discontinuation of these contraceptives were i) 247 of women had side effects with intra-uterine contraceptive device (IUCD) (34.3%), ii) 187 women could not comply with oral pills and also had side effects (25.9%), iii) 226 women discontinued condom for fear of pregnancy and lack of privacy (31.4%), iv) 31 discontinued injection because of high cost (4.3%) and remaining 2 had failure of tubal ligation (0.3%). Continuation rates of injection Net-En using a life-table method of calculation at the end of 12, 18 and 24 months were 65 percent, 53.6 percent and 48.3 percent respectively (Fig. 29). Majority of the discontinuations were due to personal reasons and lost to follow up/migration because of floating population (Table 5).

Participants were interviewed at discontinuation of injection about their views and attitudes towards this method. Over 79 percent of women were satisfied with the method (Fig. 30).

About eighty eight percent said that the 2 monthly schedule of injection is "convenient to comply". Over 89 percent of women expressed that the method should be available in our National Family Welfare Programme (NFWP) as the "bimonthly

Table 4: Contraceptive methods used prior to participation in the study (n=721).

Contraceptive Used	Number	Percent
IUCD	247	34.26
Condom	226	31.35
Oral pills	187	25.94
Injection+ Norplant	31	4.30
Other Methods (Withdrawal, Safe Period, Spermicidal)	28	3.88
Tubal Ligation Failure	2	0.28

Table 5: Reasons for discontinuation of injection Net-En.

Reasons for discontinuation	6 months %	12 months %	18 months %	24 months %
Menstrual irregularity & weight gain	8.44	4.22	2.23	0.41
Pregnancy	0.25	0.0	0.08	0.0
Personal reasons	6.2	5.13	4.8	1.99
Other medical reasons	1.99	1.16	0.33	0.66
Awaiting tubal ligation	1.65	1.9	1.49	0.91
Late for injection	1.99	0.83	0.50	0.17
Lost to follow up/migration	10.92	2.98	2.23	0.74

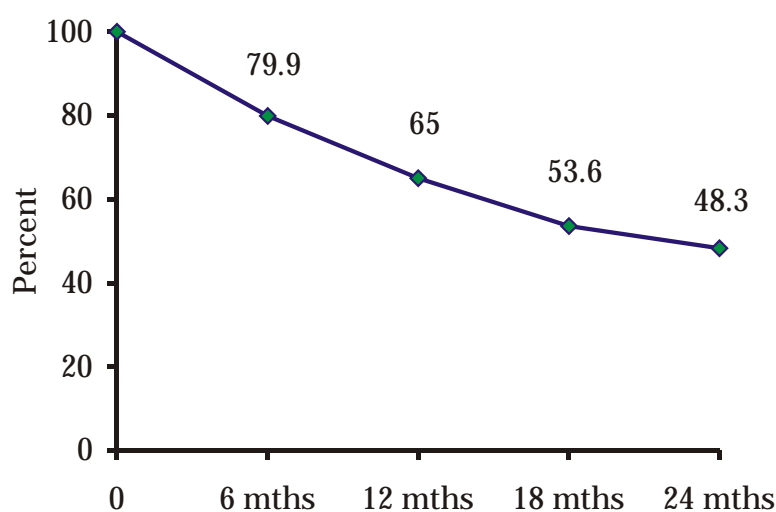


Fig. 29: Cumulative continuation rate of Net-En (women months of use=17268).

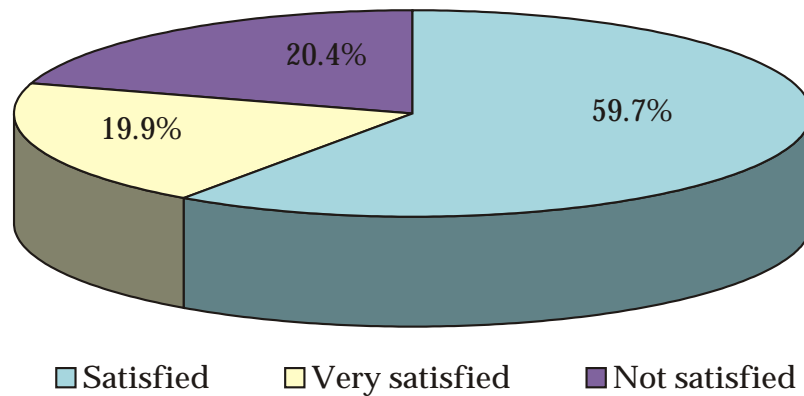


Fig. 30: How did you find injection for contraception? (n=968).

schedule is convenient as against daily intake like oral pills", "majority of women can afford and access through NFWP" and "does not have adverse effect on breast milk". About 39 percent of women expressed that they would like to use this method beyond 2 years and 219 women were given additional injection on request after obtaining permission from Institutional Ethics Committee. Over 79 percent of women said that they would recommend this method to their friends and relatives, major reasons being "one is care free for 2 months", "the method is private" etc (Fig. 31 & Table 6). About 21 percent of women had concerns about the effect of drug on return of fertility and progeny, HIV transmission through repeated needle pricks, cancer, developing male characteristics etc. These were alleviated through education and counseling.

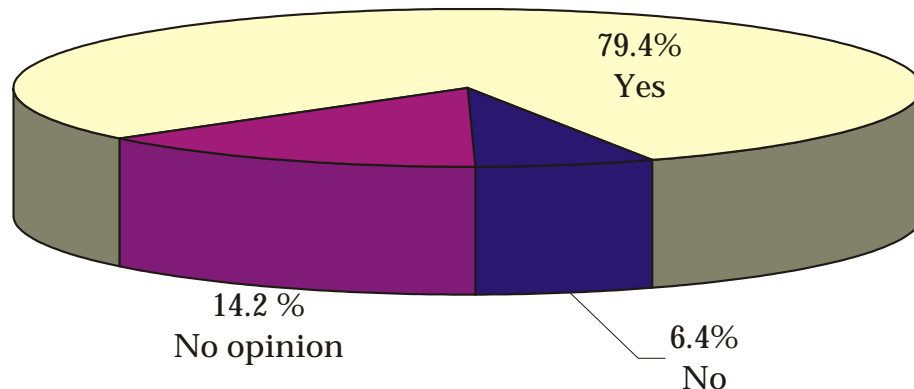


Fig. 31: Would you recommend injection to your friends/relatives? (n=968).

**Return to fertility:** Women who wished to conceive following drug discontinuation are being followed up for 12 to 24 months for return to conception. So far 123 women have reported pregnancy and of these 92 percent have conceived during first 12 months following drug discontinuation (Table 7).

Table 6: If yes, Why? (n=769).

Reasons for recommending	Number	Percentage
Care free for two months no hassle like taking daily pills	502	65.3
Better than IUCD as it is invasive procedure	132	17.2
Effective method	41	5.3
Suitable for breast-feeding women	32	4.2
Non coital dependent	27	3.5
Privacy can be maintained	16	2.1
Less pain during menses	4	0.5
No response	15	2.0

Table 7: Return to fertility (n=123).

Duration in months after drug discontinuation to conception	Number	Percentage
Up to 6 months	65	52.85
7 to 12 months	48	39.02
More than 12 months	10	8.13

#### 1.7.1 Acceptability and Continuation Rates of 2 Monthly Injectable Contraceptive-Norethisterone Enanthate: Protocol amendment DEXA study (*Funded by Ministry of Health & Family Welfare*)

In May 2005, a women's health group raised issues about Bone Mass Density (BMD) among long-term users of 3 monthly injectable contraceptive Depo-Provera and "Black Box" warning issued by USFDA on the use of injection Depo-Provera. In view of this, Institute's Ethics Committee (IEC) recommended and approved the study on bone mass evaluation by DEXA among injection Net-En users. The study has been initiated at 6 centres where DEXA facility is locally available. An additional parameter to assess BMD by DEXA has been studied among 142 users of Net-En and of these 73 active participants underwent repeat DEXA at 6 months interval. There was marginal increase in BMD at hip and significant increase at lumber spine between first and second DEXA (Fig. 32). DEXA investigation will be carried out one year after stopping the injection i.e. during recovery phase. The study is ongoing.

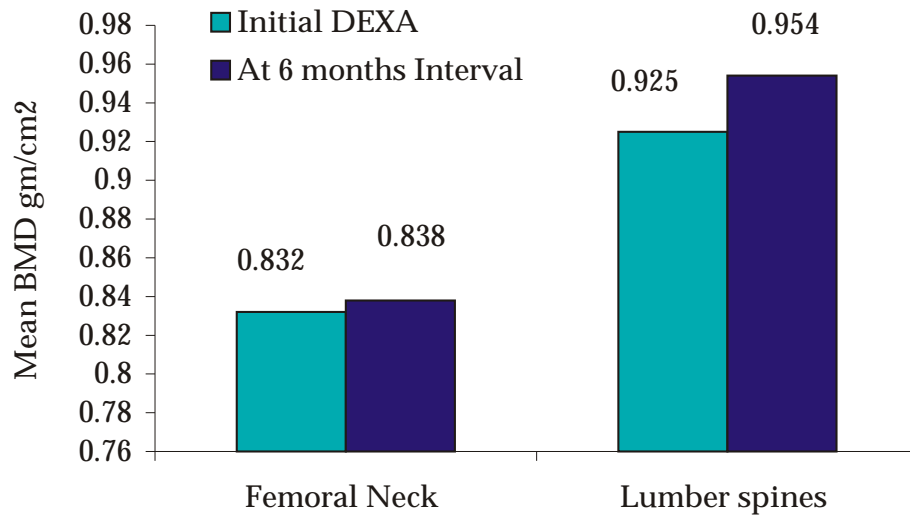


Fig. 32: BMD values by DEXA among Net-En users (n=73).

1.8 Interventions in Urban Slums for Enhancing Participation of Men in Reproductive Health (*Funded by Department of Family Welfare, Ministry of Health and Family Welfare, Government of India*)

Principal Investigator: Donta Balaiah

Project Associates: D.D. Naik, U. Iddya, Saritha Nair and P. Tapase

Collaboration: Municipal Corporation of Greater Mumbai

Duration: 2004-2007

The Program of Action of the International Conference on Population and Development (ICPD) clearly set a new agenda in 1994 on male responsibilities and participation. The programme emphasized the need to promote gender equality in all spheres of life, including family and community life, and to encourage and enable men to take responsibility for their sexual and reproductive behavior and their social and family roles. Realizing the need for involving men in the programme, the draft National Population Policy (2000) suggested a holistic approach to family welfare programme and stressed on male involvement particularly in the context of prevailing gender inequality. If men are brought into a wide range of reproductive health services in such a way that they are considered as equal partners and responsible parents, as well as clients in their own right, better outcome can be expected in reproductive health indicators such as contraceptive acceptance and continuation, safer sexual behaviour, use of reproductive health services, and reduction in reproductive morbidity and mortality. Hence, there is a need to address the reproductive health concerns of men, as also of the couple for improving the

reproductive health seeking behaviour of couples.

The overall objective of the study is to identify programme strategies contributing to effective participation for men in programmes aimed at improving reproductive health. Specific objectives are to i) study the knowledge, perception and practices among married men and married couples regarding safe motherhood and family planning to assess knowledge regarding RTI/STI and HIV/AIDS ii) to determine decision making process on issues related to safe motherhood, family planning/contraception, RTI/STI and HIV/AIDS iii) to investigate the reproductive health seeking behavior and the support they had received from their spouses; (iv) plan appropriate intervention for enabling couples to gain correct knowledge about reproductive health issues concerning men and take appropriate actions to seek and avail reproductive health services and (v) evaluate the impact of interventions on their reproductive health seeking behavior.

Three comparable slum areas were selected (on the basis of similar population, infant mortality rate and infrastructure) with the help of Municipal Corporation of Greater Mumbai after surveying 12 health post areas for the purpose of this research study. One is experimental area-1 (Mohili Village) where intervention would be addressed to husbands only, second is experimental area-2 (Bail Bazar) where intervention would be addressed to couples and third is control area (Asalfa Village) where no intervention is proposed. The ongoing government reproductive health and family welfare programmes will continue in all three-study areas. The findings presented below are the information collected from 1755 couples (both husbands and wives) during December, 2004 to March, 2005 from the three health post areas namely Mohili Village, Bail Bazar and Asalfa Village.

About three-fifth of husbands were between 19 to 39 years of age. The mean age was 37.18 years. More than one-fourth of men married before legal age at marriage i.e., 21 years. A little less than half (45.2%) of them had secondary school education. The occupational status of men indicated that 39 per cent were unskilled. Mean age of wives was 31.38 years. Forty one per cent of the wives were between 17 and 29 years of age. About 42 per cent of women had married before legal age at marriage i.e., 18 years. Half (49.2%) of women had education between upper primary to secondary education. Most (91.4%) were housewives. Income status of couples indicated that 43.2 per cent of the family had income up to Rs. 3000/- per month.

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. Almost all (98%) of husbands and 73.8 per cent of wives reported that they have discussed with their spouses about the number of

children they desired to have.

About one-fifth of wives reported that they had past history of abortion of which 12 per cent had induced abortions. About 17 per cent of those who reported induced abortion had repeated abortion (more than one time MTP). Majority (98.3%) of husbands and (96.4%) of wives had reported services utilized from a doctor whereas others had from unqualified person or self medicated at home. About 63 per cent of husbands and 66.7 per cent of wives reported that both husband and wife took decision for undergoing MTP. About 8 per cent of husbands as well as wives reported that wife alone had taken decision and 14.4 per cent of husbands and 5 per cent of wives reported that husband/in-laws/parents took decision for undergoing MTP. Most of husbands (88.4%) and wives (85.6%) reported husband accompanied wife for abortion services and rest reported either wife's mother/sister/husbands parents accompanied them (Fig. 33).

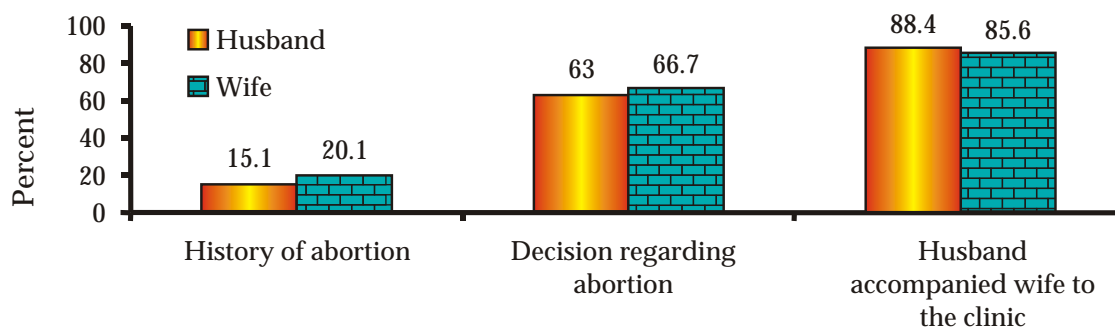


Fig. 33: Abortion and husband's involvement.

Majority of husbands (95.4%) and wives (81.7%) reported that both husband and wife decide the number of children they should have in their family. Almost all husbands (98.9%) and wives (98.3%) were aware that pregnant women should register for antenatal care. About 43 per cent of husbands and 60.7 per cent of wives reported that pregnant women should register for antenatal check-up in the seventh month whereas 22.1 per cent of husbands and 1.8 per cent of wives do not know the exact timing of registration for antenatal check-up (Fig. 34).

Most of the husbands (96.8%) and wives (90.3%) also reported that husband discussed with his wife about the antenatal check-up she needs to have. A little more than half (54%) of husbands and 47.8 per cent of wives reported that husbands accompanied wives for their antenatal check-ups, 23.1 per cent of husbands and 30 per cent of wives said other family members accompanied; 12.6 per cent of husbands and 9.8 per cent of wives said none accompanied; and rest reported that friends and neighbours accompanied. Reasons for wife not undergoing antenatal check-up were that either husband did not feel it necessary or woman herself was not interested for check-up.

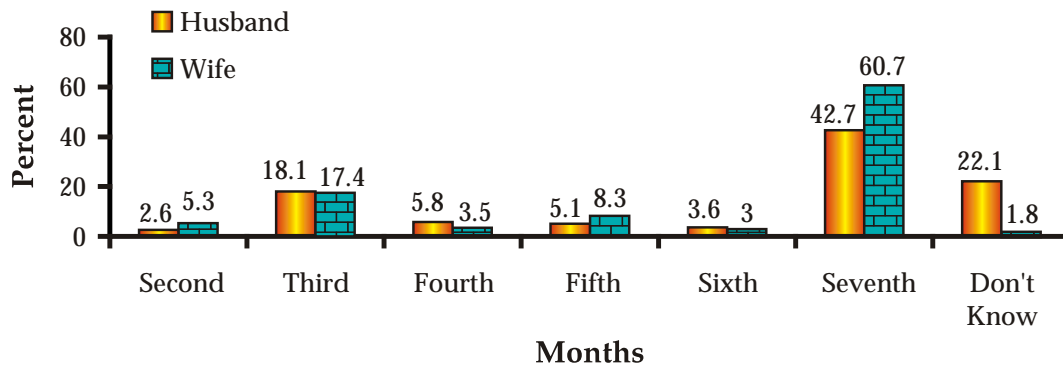


Fig. 34: In which month of pregnancy a woman should register?

Around two-fifth of husbands (41.7%) and wives (41.8%) reported that there should be two to three years of interval between two births. About 70 per cent of husbands and 42.7 per cent of wives reported that husband discussed about using spacing methods and 66.8 per cent of husbands and 53.8 per cent of wives reported that their spouses discussed about using permanent method. Majority (seventy six per cent) of couples reported that they were using one or another method of contraception. A little more than two-fifths of the husbands (43.4%) reported that their wives/themselves had undergone tubectomy and 10.1 per cent of husbands reported that they are using condom. Only one person had undergone vasectomy (Fig. 35). Regarding future intentions to use contraceptives, 30 per cent of husbands and 26.7 per cent of wives reported that they do not like to use contraceptive method in future (Fig. 36). Of these, majority of husbands (36.7%) and wives (52.5%) reported that adoption of FP is against their religion.

About one third (32%) of husbands and one-fifth (21.4%) of wives reported that they used condom in the past; one-tenth (10.1%) of husbands reported that they were currently using condom; and 57.8 per cent of husbands and 70.3 per cent of wives reported that they had never used condom. Among those who never used condom, about 36 per cent of husbands and 43.3 per cent of wives reported either that they did not feel the need to use condom or usage of condom was against religion. More than four-fifths of husbands (82.8%) and wives (82.1%) mentioned that tubectomy is better than vasectomy and 3.7 per cent of husbands and 4.3 per cent of wives mentioned that vasectomy is better than tubectomy. Both husbands and wives felt that men would become physically weak after adopting vasectomy and as men are the earning source for the family, they should not go for vasectomy. Women prefer tubectomy rather than sending husband for vasectomy, also they feel that failure of vasectomy may cause social problem too. HIV/AIDS, STIs and infertility are considered as the reproductive problems by the husbands and wives (couples). Nine out of ten husbands (97.4%) and wives (96.4%) heard about HIV/AIDS. About three-fourth (77.6 per cent) of husbands and 48.1 per cent of wives were aware of

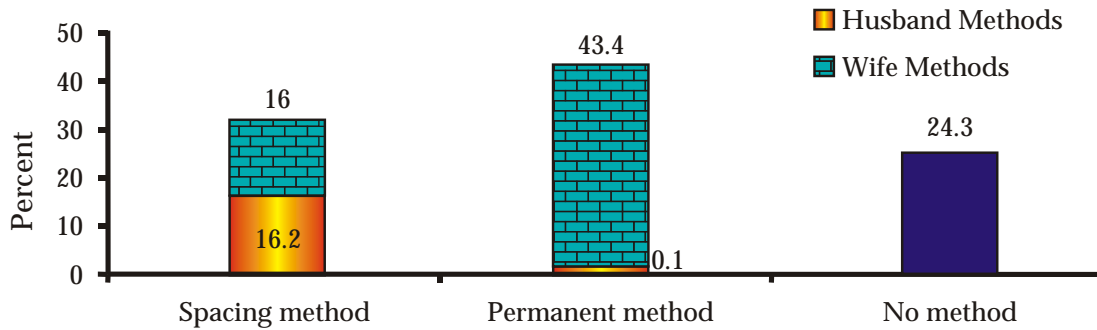


Fig. 35: Current contraceptive use reported by men.

sexually transmitted diseases. About 51 per cent of husbands and 53.1 per cent of wives were aware of infertility (Fig. 37). Little less than half of husbands and wives (49.6%) were not aware about the place, where men get treatment for infertility. Little more than half of the husband and wife reported that at public hospitals men could get treatment for STDs and HIV/AIDS. Majority of husbands (92.4%) and wives (89.2%) reported that they were aware that men and women could get infection through sexual intercourse.

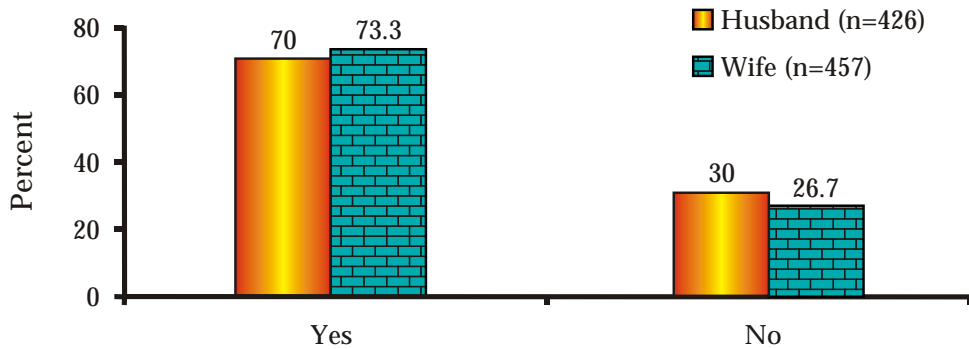


Fig. 36: Intention to use any F.P. method by husband or wife.

Regarding individual experience of reproductive problems by men during the last six months, less than one per cent of husbands 11 (0.6%) reported that they had 'genital lesion', 14 (0.8%) experienced 'genital discharge' and 12 (0.7%) experienced 'burning sensation during urination'. Of these men, 15 (83.3%) had taken treatment and 3 (16.7%) had not taken treatment. The reason for not taking treatment was reported as feeling shy and felt no need. Among those who had taken treatment, 9 (60%) sought from private hospital, 3 (20%) from government hospital and 3 (20%) used home remedies. All of the husbands and wives reported that there is no difficulty in getting treatment. Of the total 18 husbands, 14 (77.8%) reported that they have had informed their wives regarding their infection.

Regarding individual experience of reproductive problems by women during the last six months, 7 per cent of wives reported that they experienced 'abnormal

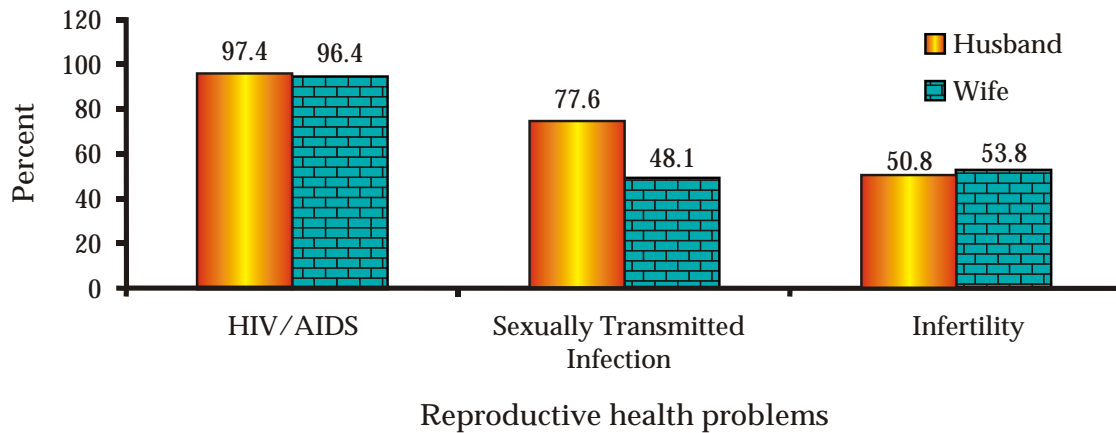


Fig. 37: Awareness about men's reproductive health problems.

discharge from vagina'; 5 per cent of wives reported that they have experienced 'Itching/redness in and around genital area'; 1 per cent of wives reported experience of 'abnormal vaginal bleeding'; less than 2 per cent of wives reported that they had experienced 'increased frequency of urine'; 3 per cent of wives reported that they had experienced 'burning sensation' during urination; about 1 per cent of wives reported that they had experienced 'lower abdominal pain'.

Majority of the husbands (99.2%) and wives (97.3%) were aware of HIV/AIDS through television, friends and neighbours, FP clinics/hospital staff radio, news paper and displayed IEC material. More proportion of wives (33.8%) than husbands (10.1%) had misconceptions about the spread of HIV/AIDS through mosquitoes and bed bugs. About 58 per cent of husbands and 37 per cent of wives were aware that condom can prevent spread of HIV (Fig. 38). A little more than three-fourth (82.8%) of husbands and 71.4 per cent of wives were aware of the test for detecting HIV/AIDS. About 36 per cent of husbands and 48 per cent of wives reported that HIV/AIDS is one of the major health problems in the area they were living. Nearly two-thirds of husbands (64.3%) and wives (62.3%) mentioned that they would give emotional support to HIV/AIDS positive persons and a few would like to help them in getting treatment. Nonetheless, one fourth of both men and women reported that they would keep distance from the person infected with HIV/AIDS.

The findings of the baseline study suggests that there needs to be intervention in the areas of a) attaining gender equality, b) preventing unwanted/unplanned pregnancy by means of promoting knowledge and correct use of contraceptives including non-scalpel vasectomy, c) promoting safe abortion practice, d) promoting early registration of couples for ANCs and safe delivery, e) promoting spacing between two children, f) enhancing knowledge regarding RTI/STI/HIV and

providing counseling to people affected g) promoting health seeking behaviour with regard to all the reproductive health problems, h) strengthening spousal communication, and i) enhancing male responsibility in reproductive and sexual health matters.

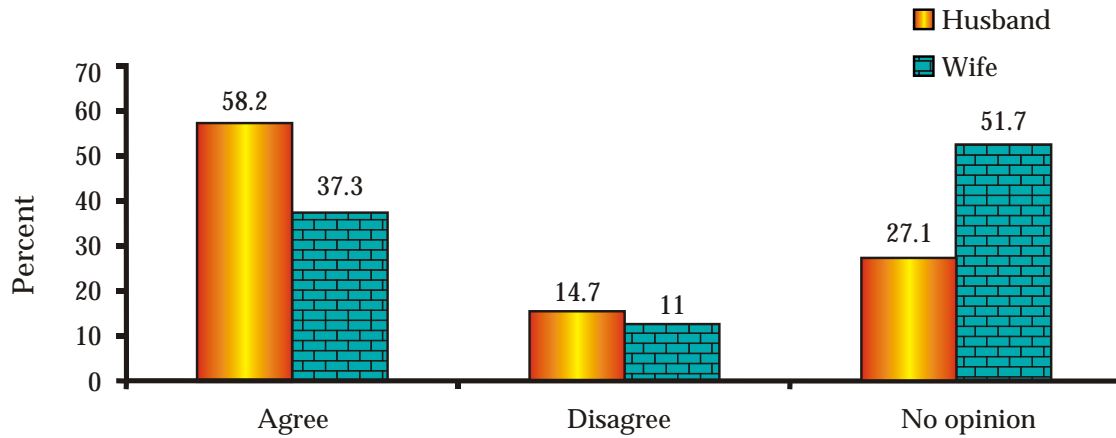


Fig. 38: Whether use of condom can prevent spread of HIV?

