

1 EXPANDING CONTRACEPTIVE CHOICES

1.1 Development of New and Improved Technologies (*Partly funded by Department of Science and Technology*)

1.1.1 Identification and Characterization of Sperm Antigens Using Multifaceted Approach

Principal Investigator : Vrinda Khole

Project Associates : A. Suryawanshi, Shagufta Khan, S. Jadhav

Duration : 1997-2010

Neonatal Tolerization – Immunoproteomics

Mammalian spermatozoon is one of the most highly specialized cell in the body with its unique functional and morphological features. Although several studies have led to identification of sperm proteins required for motility as well as interactions with the egg, many more functional proteins still remain to be identified. For a sperm protein to be considered as an ideal candidate for contraceptive vaccine, it should be sperm specific, preferably present on the surface of the sperm in order to be accessible to antibodies, highly immunogenic and must have a role in fertilization. Epididymal sperm proteins are therefore preferred targets for immunocontraception. As part of our effort to identify putative protein targets for the development of post testicular male contraceptives, we exploited unique combinatorial approach of subtractive immunization followed by proteomics to delineate epididymal maturation proteins specific to sperm head or flagella. Subtractive immunization protocol and initial characterization of proteins has been reported earlier. The mice sera not showed identical domain reactivity with human sperm. Several domain specific human sperm proteins were identified using 2D immunoblotting (Annual Report 2006-07, p 47-51).

During the reporting period, immunoproteomics experiments with rat sperm proteins were carried out using different domain specific sera. Sera showed reactivity with 31 bands with molecular masses ranging from 21 to 180 kDa in western blot using rat caudal sperm proteins. The immunoreactive bands were aligned with a corresponding gel and labeled as R1 to R31 as shown in Fig 1a. The detailed information of the immunoreactive bands is listed in Fig 1b. The 31 gel sections were manually

excised and subjected to MALDI TOF/ TOF analysis. This resulted in identification of total 30 proteins, of which 4 are being reported for the first time on sperm and another group of four proteins being reported for the first time in epididymal sperm.

Of the 4 proteins, Sperm Head protein 1 (SHP1) is on the head domain and the other three on the flagellar domain. The flagella group proteins comprised of 27 proteins of which 3 were novel proteins, Sperm Flagella protein 2 (SFP2), Sperm Flagella protein 3 (SFP3) and Sperm Flagella protein 4 (SFP4) are being reported in this study for the first time on sperm. Four proteins reported earlier in testis namely Collagen alpha-2(I) chain precursor (Colla2), Homeodomain interacting protein kinase1 (HIPK1), GTP binding protein Rab1 (RAB1) and Ubiquinol cytochrome c reductase core protein mitochondrial precursor (UQCR2) are known in testis but are being reported here for the first time in epididymal sperm, while remaining 12 have been shown to be present in testis and epididymis/ejaculated sperm.

To establish that the eight proteins identified by the proteomic analyses actually represented the immunoreactive species, peptide ELISA was carried out. Fig. 2a represents peptide ELISA results of pooled peptides of novel proteins namely R27, R1, R21, R29 with PT and PI sera of respective group (HI / HS / FI / FS). Fig. 2b represents peptide ELISA results of pooled peptides of proteins namely R18, R3, R25a, R25b with PT and PI sera of respective group (HI / HS / FI / FS). Out of these eight proteins, seven proteins showed significantly high reactivity with PI sera as compared to PT sera which confirmed the specific reactivity and validated the earlier proteomic analysis results. Only, protein R1 did not show significant reactivity with PI sera as compared to PT sera.

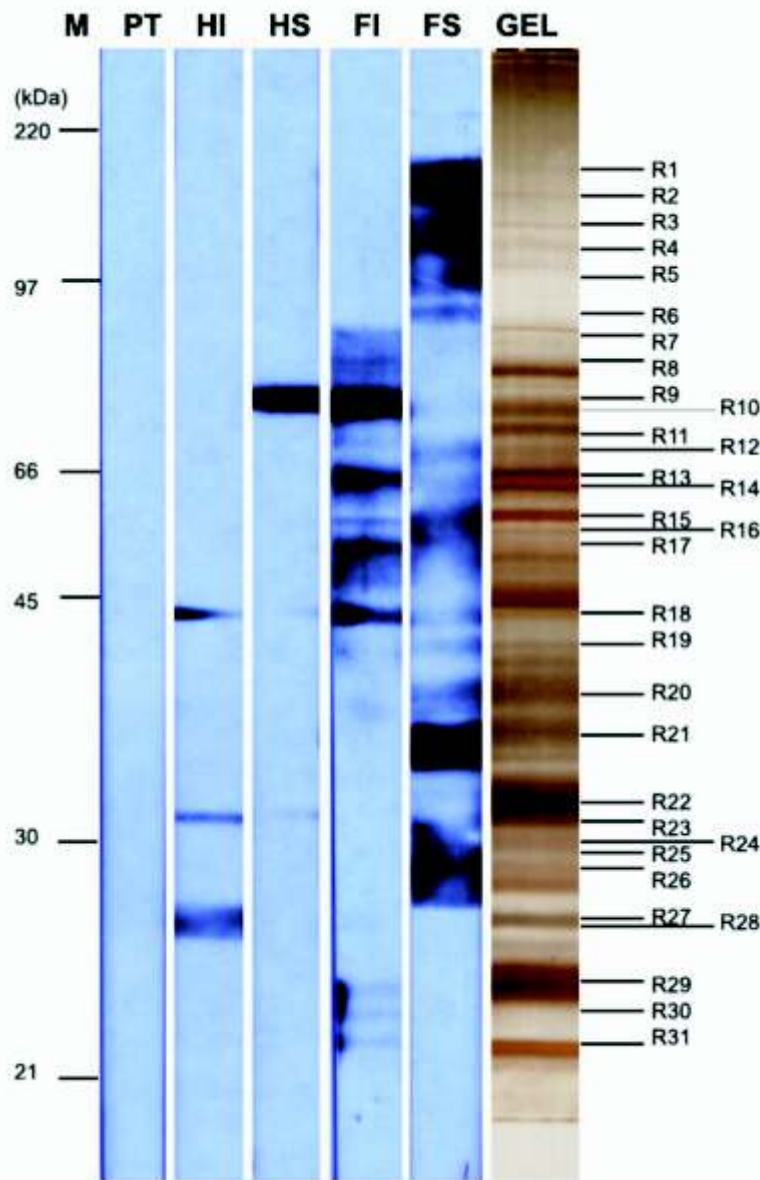


Fig. 1a

Sample ID	MW kDa	HI	HS	FI	FS
R1	182				X
R2	155				X
R3	132				X
R4	117				X
R5	99				X
R6	92				X
R7	88			X	
R8	85			X	
R9	80	X	X		
R10	77			X	
R11	72			X	
R12	69				X
R13	66			X	
R14	64				X
R15	59			X	X
R16	56				X
R17	54			X	
R18	48	X	X	X	X
R19	44			X	X
R20	41				X
R21	38				X
R22	33	X	X		
R23	32				X
R24	31				X
R25	30				X
R26	29				X
R27	27	X			
R28	26	X			
R29	24			X	
R30	23			X	
R31	21			X	
Total		4	3	13	18

Fig. 1b

Fig. 1a: Immunoreactivity of different PI sera (HI, HS, FI and FS) and PT sera using cauda epididymal sperm proteins separated on preparative 10 percent SDS-PAGE. Gel is a silver stained profile. M indicates molecular weight marker (kDa). Immunoreactive bands are marked as R1-R31 on silver stained gel. (b) Pictorial representation of immunoreactive bands (R1 to R31) with different PI sera (HI, HS, FI and FS). X indicates presence of the immunoreactive bands. MW (kDa) represents molecular mass of corresponding immunoreactive bands.

Further, the identified protein data were subjected to (GO) annotations. An annotation for subcellular distribution of each of these proteins is shown in Fig. 3. Analysis indicates that majority of the proteins show mitochondrial location.

The annotation for biological process is shown in fig 4a, 4b, 4c and 4d. The major parent category cellular process is seen in 49/67 annotable proteins indicating that majority of the

sperm proteins are involved in some sort of cellular process (Fig 4a). By breaking up the parent term into sub categories (Fig 4b), it can be demonstrated that 22 of those proteins described under the parent term of cellular process are proteins involved in cellular metabolism (Fig 4c) while 19 of them belonged to parent term transport (Fig 4d). Further breakdown of the term metabolism into its subcategories generated

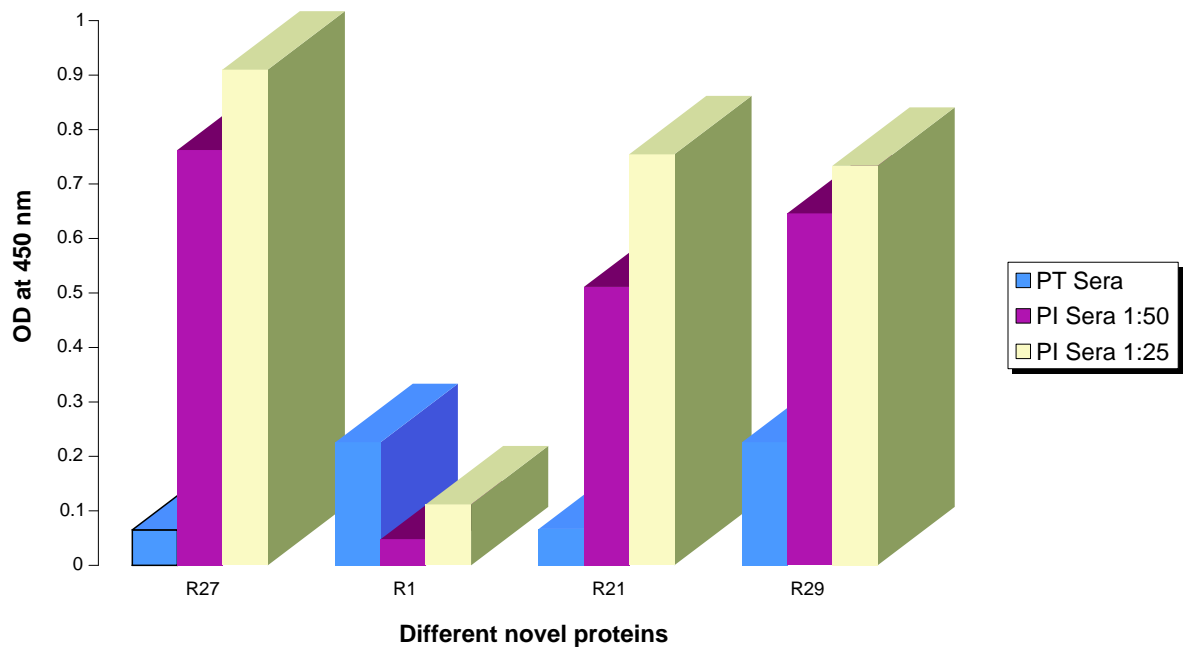


Fig. 2a: Peptide ELISA results of pooled peptides of novel proteins namely R27, R1, R21, R29 with PT and PI sera of respective group (HI / HS / FI / FS)

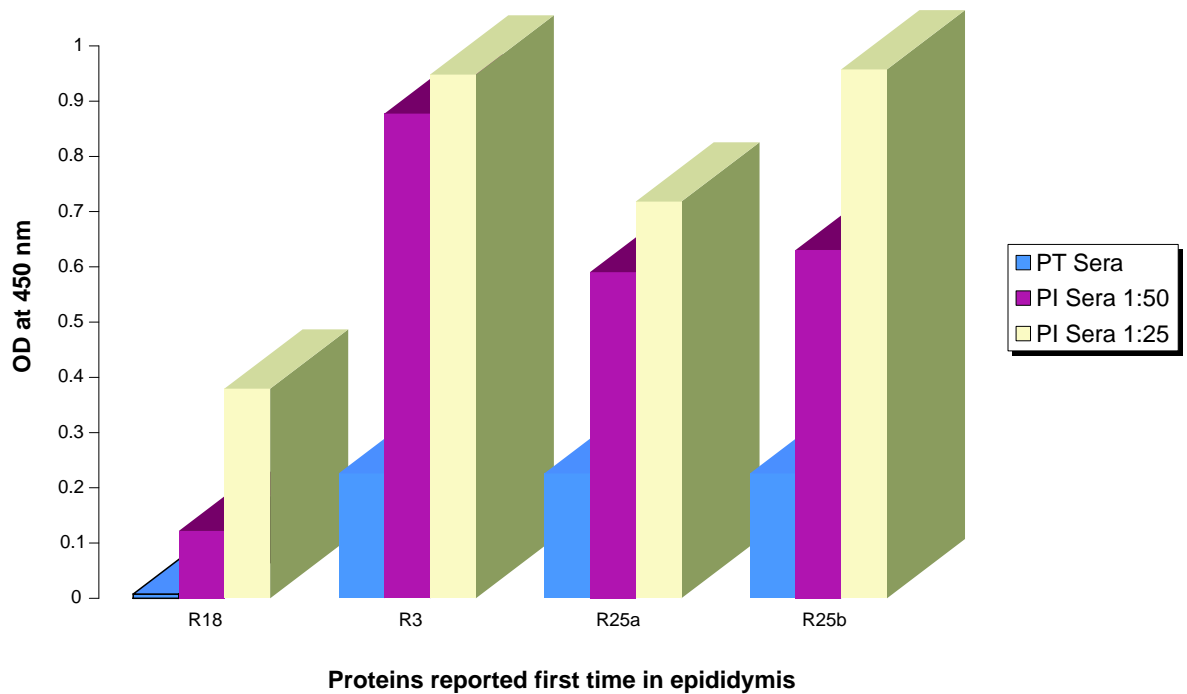


Fig. 2b: Peptide ELISA results of pooled peptides of proteins namely R18, R3, R25a, R25b with PT and PI sera of respective group (HI / HS / FI / FS)

information related to the various metabolic pathways spermatozoa may primarily use. Breakdown of the term transport into its subcategories indicated that the identified proteins may be involved in transport of ions,

hydrogen molecules or proteins or transport via intracellular or vesicle mediated mechanisms. When we analyzed the data as per pathways in which the proteins were involved, we found that indeed the sperm is a very dynamic cell with

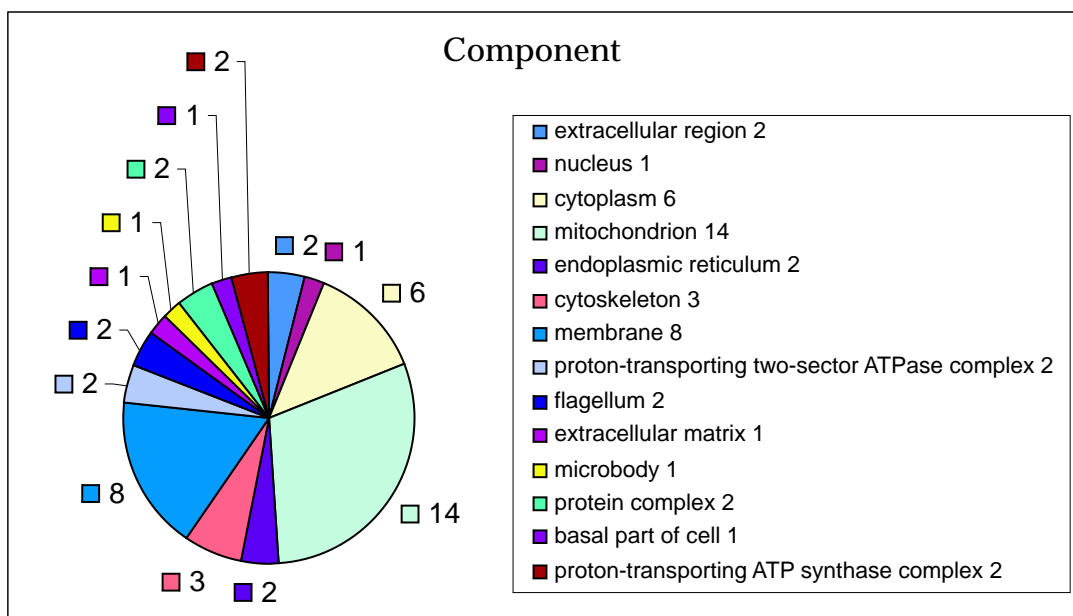


Fig. 3: GO analysis of the cellular components for the identified proteins

proteins involved in glycolysis, gluconeogenesis, carbohydrate, sugars and amino acid metabolism, oxidative phosphorylation, cell-cell communication as well as calcium signaling pathways which are all essential to make the spermatozoon a functionally mature cell.

In summary, the strategy used herein is unique and has helped in generating probes to discover several epididymal proteins on distinctly different domains of the sperm. These proteins are immunogenic in nature, conserved

in rodents and have sperm agglutinating properties. These observations indicate that the cognate proteins are highly promising potential targets for immunocontraception and need to be actively pursued. These data are a useful repository which could be exploited for mining proteins with important physiological function in sperm. Therefore the ultimate aim of our study is to identify human orthologs for the identified proteins and ascertain their functional relevance in human beings.

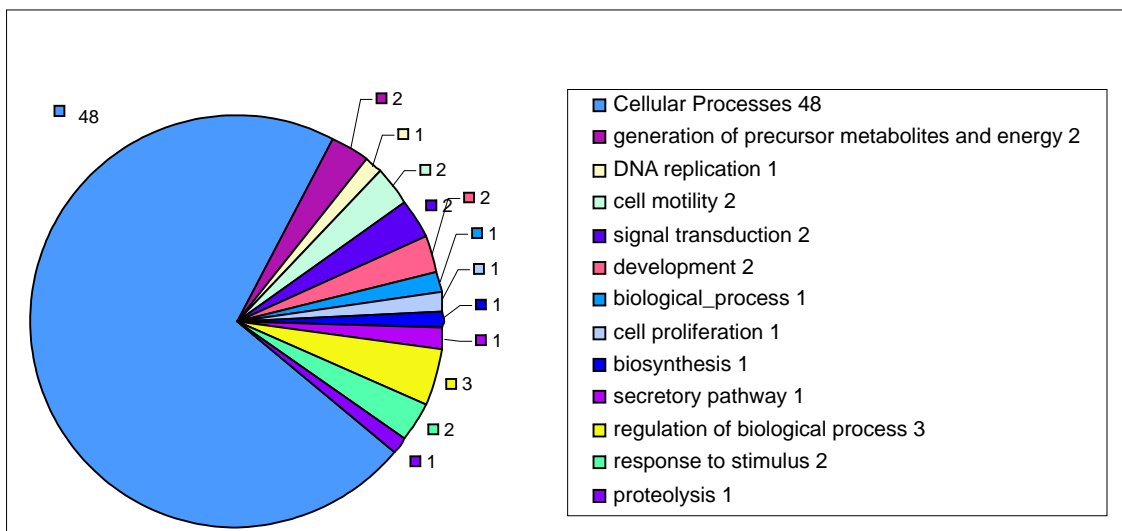


Fig. 4a: GO analysis of the biological processes for identified proteins

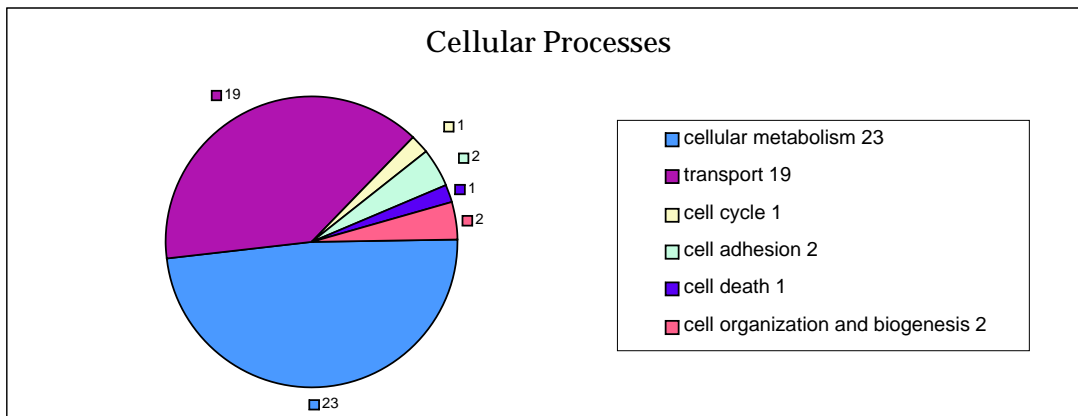


Fig. 4b: Classification of parent term cellular process.

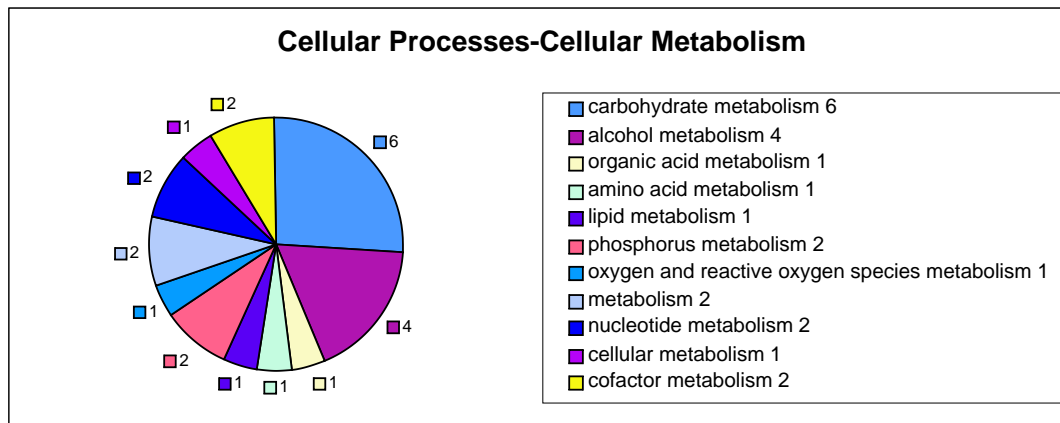


Fig. 4c: Classification of daughter term cellular metabolism.

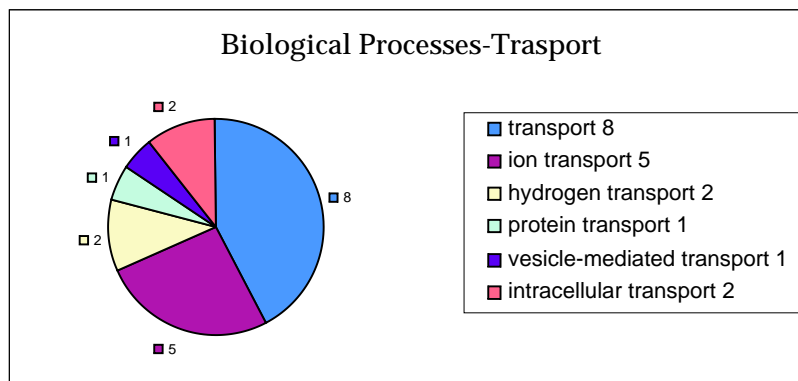


Fig. 4d: Classification of daughter term transport.

1.1.2 Role of a Novel Androgen Regulated HoxB2 Containing Gene Expressed in the Epididymis
(Funded by Indian Council of Medical Research under the Research Program on Functional Genomics)

1.1.2.1 Development of specific antibodies as a tool to study the functional role of HOXBES2 in sperm maturation

Principal Investigator : Vijaya Raghavan
 Project Associates : A. H. Bandivdekar, E. Prabakaran, R.B. Kadam, N. G. Dalvi, J. N. Tare
 Duration : 2006-2008

HOXBES2, an epididymis-specific sperm protein was identified by screening of an epididymal cDNA library using an agglutinating antibody raised against washed human sperm. The positive clone (AF255949) showed homology to the conserved region of transcription factor, HOXB2. Using a DIG-labeled DNA fragment of 581bp including the conserved HOXB2 region and a polyclonal antibody raised to the conserved region as probes; presence of a single 2.5kb transcript, epididymis-specific expression of the 30kDa HOXBES2 protein, its regionalized and cell-type specific expression, androgen dependency, postnatal-developmental expression, species conservation and its association to spermatozoa were established. The fluorescent signal of the HOXBES2 protein on sperm surface undergoes modifications in presence of various energy substrates such as ATP, cAMP and Ca²⁺. This observation is in agreement to the results obtained from the earlier studies suggesting the role of HOXBES2 in capacitated spermatozoa. The redistribution of HOXBES2 on the acrosome-reacted spermatozoa of rat, monkey and human indicates a probable role for the protein in sperm-egg interaction and its role in the maturation of sperm in the female reproductive tract. The data further indicated that HOXBES2 shares epitope similarity with embryonic HOXB2 transcription factor.

Earlier the 1.657 kb full-length cDNA sequence of Hoxbes2 gene was obtained from rat epididymis using 5'RACE-PCR approach. In order to produce the recombinant HOXBES2

protein, the ORF of the Hoxbes2 gene constructed in Pmt/v5-His-TOPO vector was induced with 0.2 mM CuSo₄ and 0.5 mM IPTG. resulting in the expression of the recombinant HOXBES2, maximally by 6 hr. The protein expressed was analyzed and confirmed by dot-blot and Western blot analysis.

A 95 percent pure, 25 amino acid peptide (N-FQNRRMKHKRQTQHREPPDGEPACP-C) corresponding to the highly immunodominant region of HOXBES2 was commercially synthesized and conjugated to Keyhole Lymphocyte Haemocyanin (KLH), for raising antibody in rabbits. The molecular mass of the peptide conjugate was confirmed by mass-spectrometry. Preimmune serum has since been collected prior to immunization from two female rabbits and was later immunized individually with 100 and 200g of the peptide conjugate subcutaneously. Following boosters at 3 weeks intervals with 200g of the peptide conjugate, post immune serum was collected at 3-4 week intervals and the antibody titers were determined by cell-free ELISA. Then the neat anti-peptide antibody was used to localize human and rat spermatozoa by indirect immunofluorescence. The anti-HOXBES2 peptide antibody detected the specific signal on the acrosome and midpiece of rat and human spermatozoa. The anti-peptide antibody also detected the recombinant HOXBES2 protein on the Western blot. These observations confirmed the earlier findings and were in agreement to observations made the using anti-HOXB2 antibody.

1.1.3 Studies with 80kDa Human Sperm Antigen (80kDa HSA) and its Synthetic Peptides for Immunocontraception

Principal Investigator : A.H. Bandivdekar
Project Associates : Vijaya Raghavan, Jacintha Pereira, Bharati Khobarekar, Sareena D'Souza, R.B. Kadam and N.G. Dalvi
Duration : 1993 – 2009

80kDa human sperm antigen (80kDa HSA) has been identified from human sperm extract by Western blot technique using serum of an immunoinfertile woman. Subsequently it is demonstrated to be sperm specific and induced immunological infertility in male and female rats following active immunization with purified

antigen. Incubation of anti 80kDa HSA antibodies with human, rat and monkey spermatozoa, *in-vitro* resulted in agglutination. Immunofluorescent studies demonstrated the localization of 80kDa HSA on head region of human, monkey spermatozoa while in the case rat the localization was predominantly on tail

region and also on head region in few sperm (Annual report 2004). 80kDa HSA expression is developmentally regulated (Annual report 2003-2004) suggesting that it could be possibly be androgen regulated. This was confirmed by evaluating the expression of 80kDa HSA in androgen supplemented chemically castrated rats. Treatment with Ethylene Diamethane Sulphonate (EDS) resulted in drastic decrease in the expression of 80kDa HSA in rat testis and epididymis (Annual report 2006-07, p 55-58) as compared to that of control animals. The decreased expression was restored following supplementation with dihydrotestosterone (Annual report 2006-07, p 55-58). The data suggest androgen dependent expression of 80kDaHSA in rat testis and epididymis.

Partial N-terminal amino acid sequence of 80kDa HSA (Peptide NT) and its peptides obtained by digestion with endoproteinase Lys-C (Peptides 1, 2, 3 and 4) and with endoproteinase Glu-C (Peptides 5 and 6) did not show sequence identity with any of the known protein sequences in the database. The peptides were synthesized, conjugated to Keyhole limpet haemocyanin (KLH) and used as an immunogen to raise the antibodies in rabbits. Passive administration of antibodies to peptides NT, 1, 2 and 4 caused agglutination of rat epididymal spermatozoa with loss of motility and these rats failed to impregnate normal females. Passive administration of these anti-peptide antibodies in female rats also resulted in infertility. Antibodies to peptides NT and 1 were found to be most effective in inhibiting fertility. Passive administration of 10 and 40µg of purified IgG fraction of antibodies to peptides NT and 1 also resulted in dose dependent inhibition of fertility

in female and male rats.

Active immunization of male rabbits with KLH conjugated peptides 1 and NT resulted in agglutination of ejaculated spermatozoa with complete loss of motility and these rabbits failed to impregnate the normal females. These rabbits regained fertility with decline in antibody titer, following cessation of immunization (Annual Report 2001-2002, p 28). Further active immunization of male marmosets with KLH conjugated peptide-1 impaired motility and six out of the seven marmosets having antibody titer greater than 1:400 failed to impregnate the normal females. All these animals regained fertility with decline in antibody titer, following cessation of immunization (Annual Report 2004-05, p 38, 39).

Electron microscopic studies in whole sperm mount demonstrated localization of 80kDa HSA on the surface of head and neck-midpiece region of human sperm on the surface of head and tail region of rat sperm and on the surface of head region of monkey spermatozoa. Further electron microscopic studies on sperm ultra sections using antibodies to 80kDaHSA demonstrated the strong immunoreactivity in the nucleus and weak immunoreactivity in the postacrosomal ring of human spermatozoa (Fig. 5), in marmoset the immunoreactivity was observed only in the nucleus (Fig. 6)

The data suggest that 80kDa HSA expression is androgen regulated. The peptide-1 of 80kDa HSA is immunogenic active immunization with this synthetic peptide resulted in reversible infertility in marmosets. Thus suggesting the potential of 80kDa HSA and its synthetic peptide-1 as the candidate for development of antifertility vaccine.

1.2 Studies with FSH Binding Inhibitor: Functional Significance of FSH Modulators from Follicular Fluid in Ovarian Pathophysiology (*Funded by CONRAD*)

Principal Investigator	:	Tarala D Nandedkar
Project Associates	:	Swati Kulkarni, Serena D'Souza and Smita Mahale
Duration	:	2006-2009

Octapeptide (OP) a partial N-terminal eight amino acid sequence (AESNEDGY) of follicle stimulating hormone binding inhibitor has shown to possess similar biological activity as

the native peptide. In vitro, OP inhibited the binding of FSH to its receptors on granulosa cells in a radio receptor assay. A dose dependent decrease in FSH induced progesterone (P4)

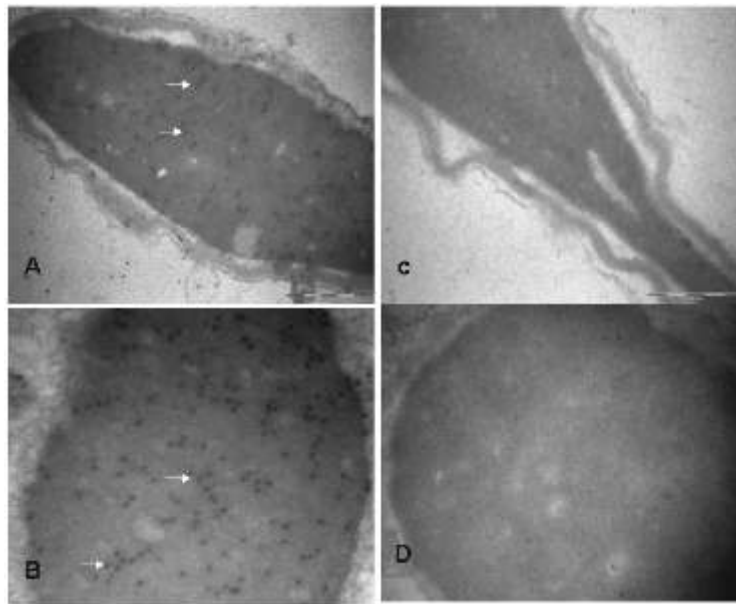


Fig. 5: Ultrastructural localization of 80kDa HAS in human sperm. It is predominantly localized in the nucleus (A, B). No immunoreactivity was observed with normal rabbit sera (C, D).

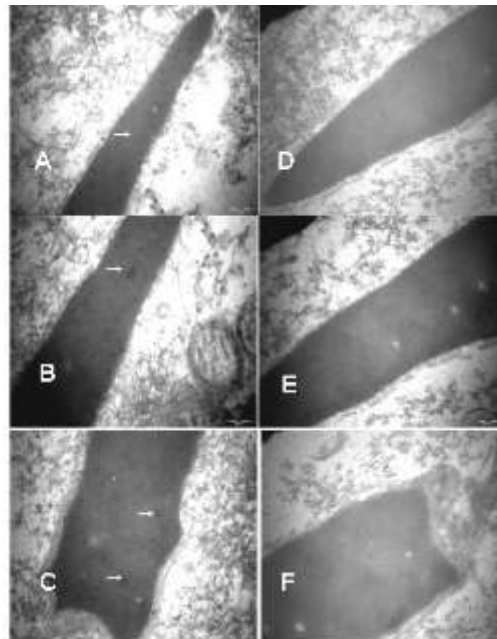


Fig. 6: Ultrastructural localization of 80kDa HSA in marmoset sperm. It is localized in the nucleus (A, B, C). No immunoreactivity was observed with normal rabbit sera (D, E, F).

secretion was observed when granulosa cells were cultured in presence of OP.

Flow cytometric analysis of follicles from normal, atretic and OP treated groups revealed that apoptosis occurs in granulosa cells of atretic and OP treated follicles. It was observed that in atretic follicles it occurred predominantly by mitochondrial pathway and in OP treated follicles by Fas – FasL pathway. These studies were supported by the immunohistochemical localization of pathway specific markers (Annual

Report 2006-2007, p 62-65). Because of this difference in initiation of apoptosis, an attempt was made to study the morphological changes at the ultrastructural level.

Immature female Swiss mice (21 – 23 day old) bred in our institute were used for the study. The animals (n = 3 / group) from Group I were injected with 10 IU eCG and autopsied after 48 hrs while those from Group II were injected with 10 IU eCG and autopsied after 72 hrs to procure ovaries predominantly containing normal and

atretic follicles, respectively. The animals in treated group (Group III) were primed with 10 IU eCG and administered with dose of 100 g of OP post eCG injection and autopsied 48 hrs after the eCG injection.

The ovaries from all the groups were fixed in modified Karnovsky's fluid, processed and embedded in Araldite resin (Pelco International, CA, USA). Ultrathin sections (60-70 nm) were stained with uranyl acetate and subsequently contrasted with lead citrate. The grids were viewed in the transmission electron microscope (Philips Tecnai).

Three grids per group were scanned and at least 6 granulosa cells of each cell type based on their location namely - antral, cumulus and basal - granulosa cells of normal, atretic and OP treated groups were compared by statistical

analysis. Changes in oocyte and oocyte - granulosa cell interaction were also observed and analyzed.

The transmission electron microscopic examination of the ovarian tissue from atretic and treated groups revealed a change in gross morphology exhibiting characteristic features of apoptosis like membrane blebbing, chromatin condensation and vacuolization (Fig. 7). There was no significant change observed in the nucleus to cytoplasm ratio in all the 3 groups but it was observed that the average size of the atretic antral granulosa cells decreased significantly ($p < 0.005$) as compared to that in the normal group (Table 1). In the OP treated group cumulus granulosa cells showed a significant decrease ($p < 0.05$) in cell size (Table 1).

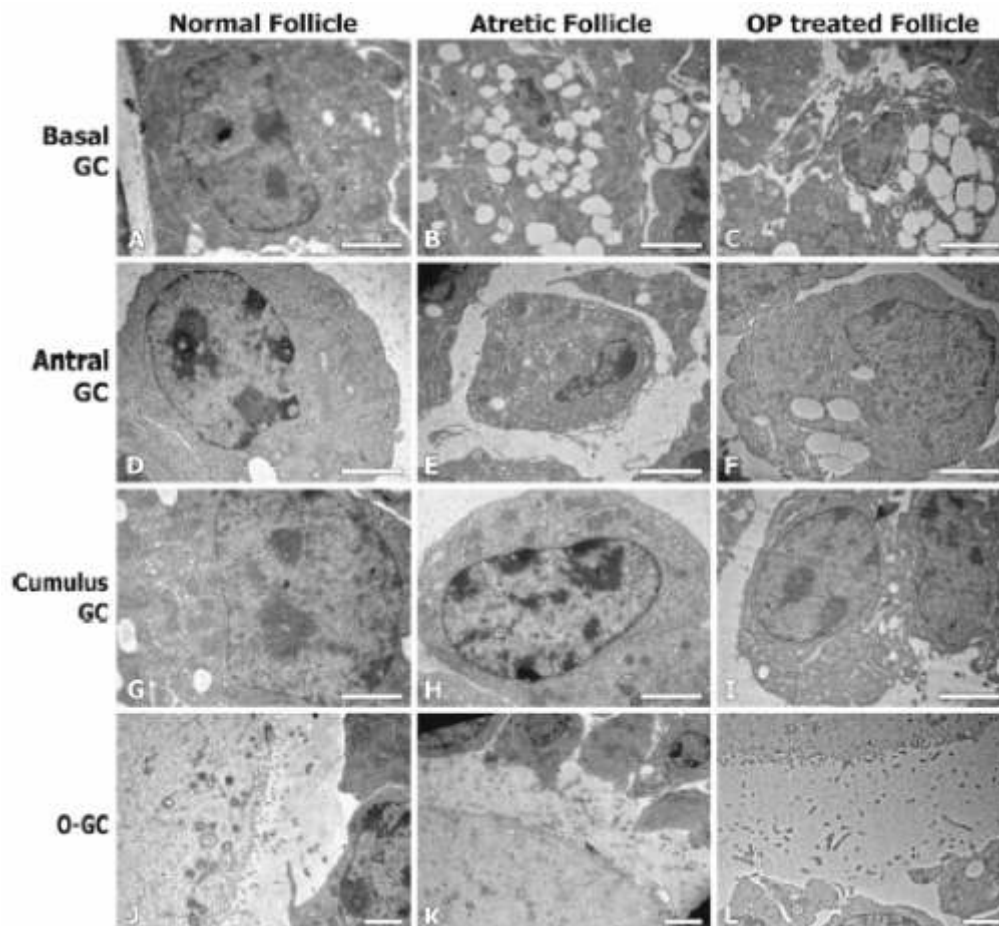


Fig. 7: Representative ultramicrographs of basal (A-C), antral (D-F), and cumulus (G-I) granulosa cells and oocyte-granulosa cell interaction (J-L) in follicles from normal and atretic- and OP-treated ovaries of mice (O, oocyte; GC, granulosa cells Q24). Characteristic apoptotic features like the intense vacuolation and condensation of the nucleus can be observed in basal granulosa cells of atretic- and OP-treated follicles. Condensation of nucleus can also be observed in antral granulosa cells of atretic follicles. Bar = 2000 nm.

A significant decrease in size of mitochondria was observed in cumulus granulosa cells of atretic group ($p < 0.005$) while in antral granulosa cells of OP treated group compared to normal group ($p < 0.005$).

granulosa cells when cultured in the presence of OP.

A thickened zona pellucida was also observed in the OP-treated group, which may possibly be caused by lipid accumulation as

Table 1: Comparison of zona pellucida thickness, oocyte diameter, nucleus : cytoplasm ratio and cell size from normal, atretic and OP treated ovarian follicles as observed by transmission electron microscopy.

	Normal	Atretic	OP treated
Zona pellucida thickness(um)	2.87 ± 0.06	3.26 ± 0.17	4.96 ± 0.47*
Oocyte diameter (um)	46.37 ± 1.45	45.36 ± 0.65	46.56 ± 2.00
Nucleus / Cytoplasm ratio			
Antral GC	0.45 ± 0.02	0.39 ± 0.06	0.30 ± 0.04
Cumulus GC	0.44 ± 0.03	0.48 ± 0.02	0.42 ± 0.05
Basal GC	0.50 ± 0.04	0.42 ± 0.05	0.46 ± 0.05
Cell size			
Antral GC	47.92 ± 2.77	26.97 ± 4.04**	50.66 ± 6.20
Cumulus GC	38.10 ± 5.47	29.54 ± 5.02	37.64 ± 3.59
Basal GC	51.64 ± 3.71	50.69 ± 3.37	40.74 ± 2.69*

(* $p < 0.05$, ** $p < 0.002$)

A significant decrease ($p < 0.05$) in the number of mitochondria was observed in cumulus granulosa cells of atretic group and basal granulosa cells of both atretic and OP treated group.

Interestingly, a significant ($p < 0.05$) increase in the width of the zona pellucida of the ovaries OP treated follicles was observed. It led to a remarkable loss in the transzonal oocyte – granulosa cell communications (TZP's). This also corroborated with the striking shrinkage of cumulus granulosa cells and also the decrease in mitochondrial number. This observation also corroborates our earlier findings that there is a drastic decrease in progesterone (P4) secretion by

suggested by Guraya (1966). In conclusion, these studies showed that the difference in the pattern of apoptosis in the atretic- and peptide-treated groups may be caused by the difference in initiation of the apoptotic pathway, spontaneously by deprivation of hormone in the atretic group, and by induction of the membrane receptor pathway in the peptide-treated groups. Moreover, the ultrastructural studies also supported the difference in the initiation of the apoptotic pathways being antral granulosa cell atresia in the atretic group and cumulus granulosa cell atresia in the peptide-treated groups.

1.3 Assessment of Safety, Efficacy and Acceptability of Existing Methods of Fertility Regulation

1.3.1 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, Lalita Savardekar, Chitra Thosar, Neha Minde, Shalini Baji, Pratibha Kokate, Deepika Belekar, P.Tapase and Kranti Paradkar

Investigators and Participating Centers : H. Saini, S.S.G.S. Medical College, Baroda
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J Mallick, S.C.B. Medical College, Cuttack
A. Bhargava, S.M.S. Medical College, Jaipur
T K Ghosh, IPGMCR & SSKM Hospital, Kolkatta,
V. Salvi, KEM Hospital, Mumbai
C Doifode, C. Sarodey Govt. Medical College,
Nagpur , S. Salhan, Safdarjung Hospital,
New Delhi

Duration : 2002-2007

This multicentre study had been initiated at the initiative of Department of Family Welfare, Government of India. The objectives are i) to assess user acceptability / continuation rate of injectable contraceptive-Norethisterone Enanthate (Net-En); ii) to evaluate the incidence of menstrual irregularities and other side effects; iii) to assess socio-behavioural aspects of the users and compare with different regions and cultural settings; iv) to study the return of fertility following discontinuation of injection.

During the reporting period, a 2 year follow-up of women for return of fertility and analysis of other variables like menstrual pattern have been completed after enrolling 1209 women across India. Women desiring pregnancy or who did not accept contraceptives in the clinic after discontinuation of injection (after the wash out period of drug) were followed up every 3 months for return of fertility over a period of 2 years. Thus a total of 150 women who desired pregnancy were eligible. The pregnancies were confirmed either by urine pregnancy test or

ultrasonography. Over 75 percent of the women reported pregnancy within 9 months of drug discontinuation. Although, conception was delayed in about 12 percent of women beyond 1 year following discontinuation of injection, no woman had requested for the investigations of delayed conception. These women conceived between 13 to 23 months (Fig.8). Of the 150 women, 135 continued pregnancy till term and had normal babies at birth, 3 women had first trimester spontaneous abortions, whereas 12 women underwent surgical abortions (MTP). These women wanted to continue injections beyond study period hence did not accept other contraceptives available in the programme. They underwent concurrent tubal ligation. Had we continued injections in these women, they would not have had unplanned pregnancies. There was no difference in timing of conception between women who discontinued injection for planning pregnancy compared to the women who discontinued for other reasons as stated in Table 2.

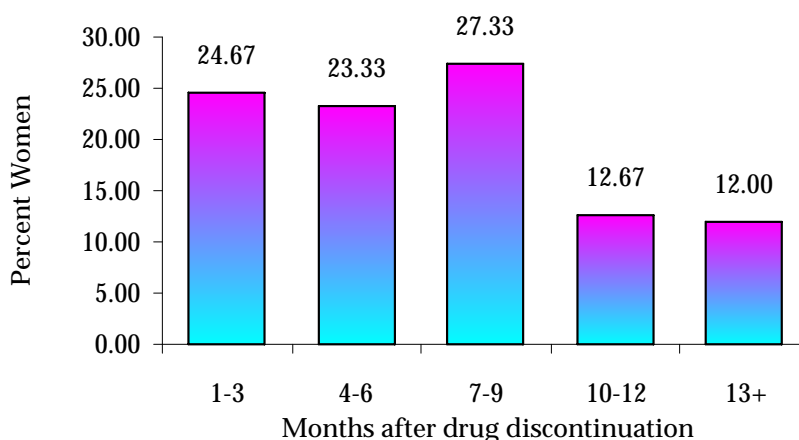


Fig. 8: Return of fertility following drug discontinuation (n=150)

Table 2: Return of fertility and reasons for discontinuation of injection Net-En (n=150)

Reasons for discontinuation of injection	Number (%)	Return of fertility in months following drug discontinuation
Desired pregnancy	109 (72.7)	1 to 23
Personal reasons	18 (12.0)	1 to 19
Irregular / Prolonged Bleeding	8 (5.3)	1 to 9
Other medical reasons	6 (4.0)	1 to 9
Amenorrhoea	4 (2.7)	1 to 19
Late for injection	3 (2.0)	7 to 19
Weight loss / Weight gain	2 (1.3)	4 to 9

Menstrual Pattern among Injection Net-En Users:

“A *bleeding/spotting episode* is defined as *requiring sanitary protection*”.

Each woman had maintained the record of bleeding episodes on menstrual diary card as

Infrequent Bleeding- 1-2 bleeding or spotting episodes

Regular Pattern – 3-4 episodes of bleeding or spotting each lasting about 5-7 days

Amenorrhea - No bleeding / spotting days during the reference period.

National Institute for Research in Reproductive Health
Menstrual Diary Record

Name :		Centre No.		Serial No.																															
Year	Day Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31			
									I									●	●	●	●	●	●												
									X	X	X	X																							

Symbols : B-bleeding, P-profuse bleeding, S-spotting, I-injection, to be transferred on to the menstrual card from subjects diary card
Symbols for Subject : X-bleeding, XX-profuse ● spotting

Checked by Dr. _____ Signature _____

instructed. A sample menstrual diary card is shown below.

For the analysis of the menstrual pattern, a 90 days reference period was considered and the statistical programme was developed based on the WHO guidelines by Belsey et al (1986) and modified as per Datey et al (1995).

The following definitions as per WHO guidelines for describing various menstrual patterns were considered

Frequent Bleeding – More than 4 bleeding /spotting episodes during the reference period

Thus a total of 5666 reference periods were studied during a 2-year injection use. It was observed that throughout the study period the infrequent bleeding (oligomenorrhoea) pattern was most commonly seen whereas regular or acceptable bleeding pattern was observed among 13.4 percent and 12.9 percent of women during the reference period at 1 and 2 years respectively. The frequent and prolonged bleeding pattern was observed only in 6.2 percent and 4.8 percent of women during these reference periods respectively. There was increase in amenorrheic

pattern at the end of 2 years of injection use (37.7%) compared to 27.2 percent during the reference period at 1 year (Fig. 9). It was observed that infrequent bleeding pattern was well

accepted by the women after effective counseling.

The study was completed in March 2008 and the final report was submitted to the Ministry of Health and Family Welfare, New Delhi.

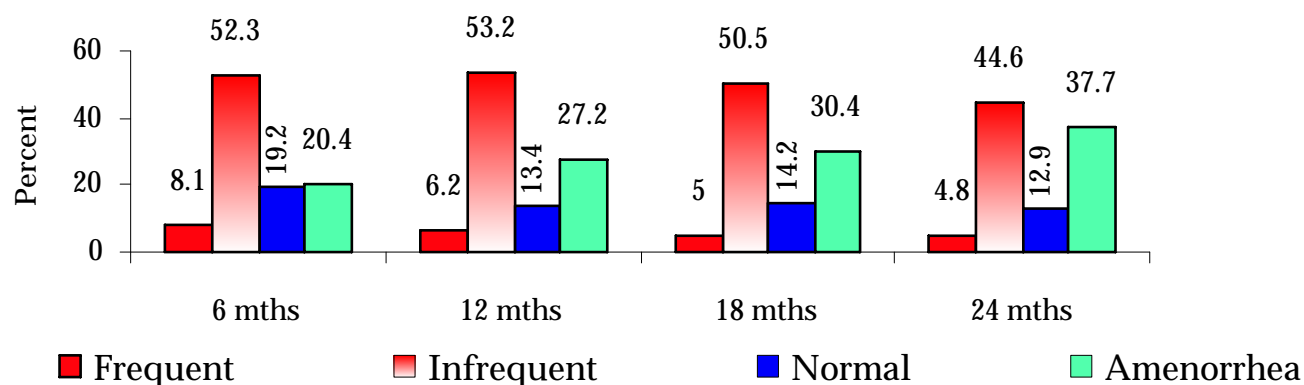


Fig. 9: Percentage of different Menstrual Pattern in a two year study period.

1.3.2 Acceptability and Continuation Rates of 2 Monthly Injectable Contraceptive: Norethisterone Enanthate: Protocol Amendment for Bone Mass Density (BMD) Evaluation by DEXA. (Funded by Ministry of Health and Family Welfare)

Principal Investigator	:	Shanta Chitlange
Project Associates	:	Kamal Hazari, D. Balaiah, Lalita Savardekar, Chitra Thosar, Neha Minde, Shalini Baji, Pratibha Kokate, Deepika Belekar, P.Tapase and Kranti Paradkar
Investigators & Participating Centers	:	H. Saini, S.S.G.S. Medical College, Baroda D Kalavalli, RSRM Govt. Hospital, Chennai J Mallick, S.C.B. Medical College, Cuttac A. Bhargava, S.M.S. Medical College, Jaipur T K Ghosh, IPGMCR & SSKM Hospital, Kolkatta, V. Salvi, KEM Hospital, Mumbai C Doifode, C. Sarodey Govt. Medical College, Nagpur, S. Salhan, Safdarjung Hospital, New Delhi
Duration	:	2005-2007

The study was initiated at 6 participating centres viz. Baroda, Chennai, Jaipur, KEM hospital Mumbai, New Delhi and NIRRH Mumbai where DEXA facility was locally available after the Institute's Ethics Committee (IEC) approval. During the reporting period, final DEXA scans were done one year after stopping the injection Norethisterone Enanthate (Net-En) among 109 participants. BMD was measured at femoral neck and lumbar spine. It was observed that mean BMD values at both sites

were significantly higher amongst those who discontinued the injection as compared to users who continued with the injection. (Fig. 10).

Since baseline values (before initiation of injection) of BMD could not be assessed in these women, it was necessary to compare BMD with those who did not use any hormonal contraceptives in the past. Therefore, a total of 59 healthy women matched with respect to age and parity with those of users of injection were enrolled as a control group for DEXA study.

The participants for control group were selected from the Institute's family welfare clinics after obtaining IEC approval and an informed written consent from the participants. Lactating women and those who desired pregnancy were excluded from the study. Observations showed that there was no significant difference in mean BMD among users of injection compared with control group (Fig. 11).

DEXA study findings revealed that there was no association between the increased duration of the injection and mean BMD at both the sites. Although, a significant increase in mean BMD was observed amongst those who discontinued the injection at both the sites ($P < 0.001$), as seen in Fig.10, it could be due to the effect of counselling regarding good nutrition, calcium supplements and exercise before and after the participants underwent DEXA scans.

The study is complete and the report submitted to the Ministry of Health and Family Welfare, New Delhi.

Observations of DEXA study:

There was no correlation between duration of injection use and mean BMD. There was positive correlation between Body Mass Index (BMI) and BMD. Women with BMI of 25 and above had significant ($p < 0.001$) higher BMD at both the sites compared to women having low BMI of less than 20. There was inverse correlation between parity and BMD. Mean BMD was significantly higher (0.886 gm/cm^2 $p < 0.05$) among women having 1 or 2 children compared with women having 3 or more children (0.769 gm/cm^2). There was no significant difference in mean BMD among injection users compared with control group.

1.4 Enhancing the Role and Responsibilities of Men in Reproductive Health

There has been a general agreement among countries in the world, particularly after the International Conference on Population and Development (ICPD) held at Cairo in 1994, that

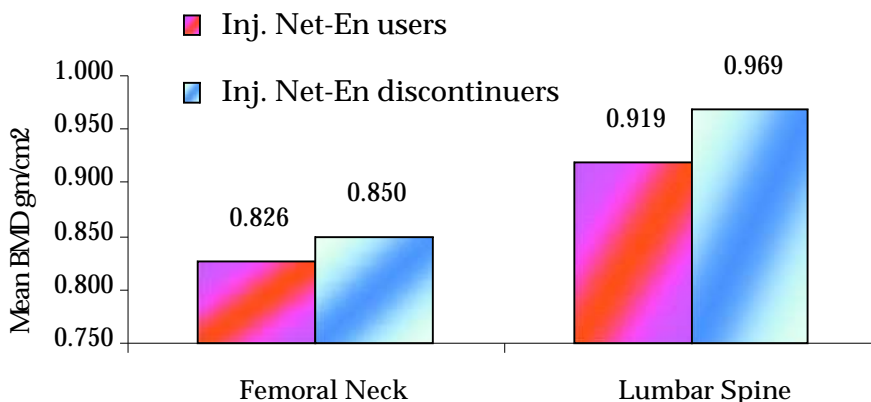


Fig. 10: BMD values by DEXA among injection Net-En users compared with injection discontinuers (n=109)

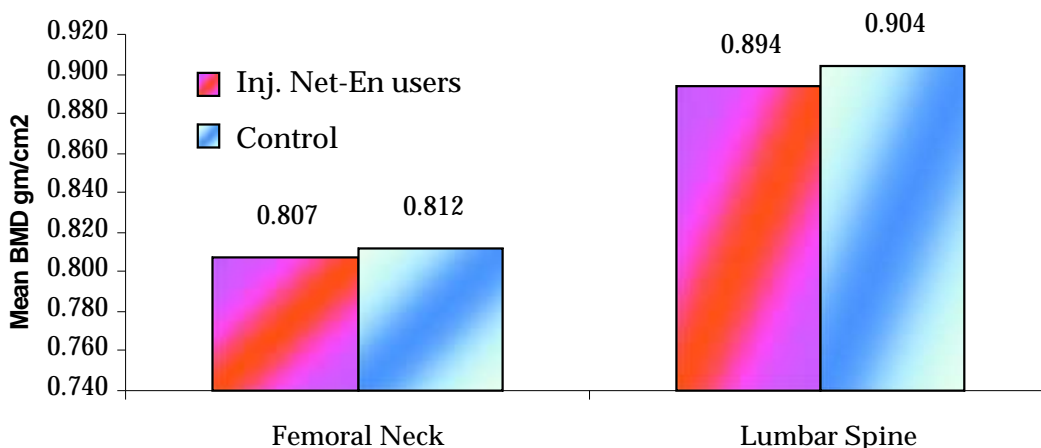


Fig. 11: BMD values by DEXA among injection Net-En users compared with control (n=59)

there is a need for ensuring participation of men in family planning and reproductive health with a view to promote gender equality, sharing of reproductive responsibilities and also to meet men's own reproductive health needs. In India, the National Population Policy, the Reproductive and Child Health (RCH) I and II programmes envisages the necessity of male involvement in improving the reproductive health of men as well as women. The major emphasis is on improving vasectomy and involving men in safe motherhood. If men are brought into a wide range of reproductive health services in such a

way that they are supported as equal and responsible parents, as well as clients in their own right, better outcomes are expected in reproductive health indicators such as contraceptive acceptance and continuation, safer sexual behaviours, 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 couples for improving the reproductive health seeking behaviour of couples.

1.4.1 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	:	D. Balaiah
Project Associates	:	D.D. Naik, U. Iddya, Saritha Nair and P. Tapase
Collaboration	:	Municipal Corporation of Greater Mumbai
Duration	:	2004-2007

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 of the study 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 RTIs/STIs and HIV/AIDS; determine decision making process on issues related to safe motherhood, family planning, RTIs/STIs and HIV/AIDS; investigate the reproductive health seeking behavior and the support they had received from their spouses; (ii) 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 (iii) evaluate the impact of interventions addressed to married men and married couples 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

(MCGM) after surveying 12 health post areas for the purpose of this research study. One is area-1 (Mohili Village) where intervention would be addressed to husbands only, second is 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 information presented below is regarding the ongoing intervention in the intervention area-1 (Mohili Village) and intervention area-2 (Bail Bazaar).

The findings of the baseline study pointed out the need for interventions 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 RTIs/STIs and HIV/AIDS and providing Counselling 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.

Accordingly, various interventions programmes have been carried out for husbands only in intervention area-1 (Mohili Village) and for couples in intervention area-2 (Bail Bazar) since September 2005. The intervention programmes have been conducted through Information, Education, Communication and Counselling (IEC). Services provided at the MCGM health posts have been supported by the staff from the institute. Several gender cross cutting issues were addressed through various programmes. Joint decision-making process in each of the issues concerning reproductive health was promoted. This has helped in integrating this component in various intervention programmes and thereby improving couple communication and involving men in the decision-making process. Intervention programmes carried out for husbands only in experimental area-1 (Mohili Village) and for couples in experimental area-2 (Bail Bazar) during the period April 2007 to March 2008 are detailed below. These programmes as a part of the project activity address each of the issues identified at the baseline survey and respective services provided.

Intervention programmes

Group meetings

Information on safe motherhood, family planning, RTIs/STIs and HIV/AIDS were provided in the smaller group meetings. In area - 1, 63 group meetings were organized for husbands for which, 687 husbands took part. Whereas, in area - 2, 27, 28 and 23 meetings were organized for husbands, wives and couples respectively for which, 147 husbands, 192 wives and 105 couples participated respectively.

Inter-personal communication (IPC)

Husbands from Mohili Village, as well as husbands, wives and couples from Bail Bazar were contacted personally and information on four issues (i.e., safe motherhood, family planning and contraception, RTIs/STIs and HIV/AIDS) of reproductive health was provided to them. Information on Antenatal Care (ANC)

was provided to 103 husbands from Mohili Village and 11 husbands, 22 wives and 17 couples from Bail Bazar. Information on family planning was provided to 126 husbands from Mohili Village and 47 husbands, 59 wives and 62 couples from Bail Bazaar. Information on correct use of condom was provided to 126 husbands from Mohili Village and 47 husbands, 59 wives and 62 couples from Bail Bazar. Information on non-scalpel vasectomy (NSV) was provided to 42 husbands from Mohili Village, and 12 husbands, 31 wives and 5 couples from Bail Bazaar. Information on RTIs/STIs was provided to 281 husbands from Mohili Village and 104 husbands, 130 wives and 112 couples from Bail Bazaar. Information on HIV/AIDS was provided to 56 husbands from Mohili Village and 46 husbands, 96 wives and 21 couples from Bail Bazaar.

Educational programmes on reproductive health issues

Audio-visual programmes were organized for providing information on safe motherhood, family planning, RTIs/STIs and HIV/AIDS. Ten programmes were organized for husbands in Mohili village for which 192 husbands attended.

IEC material (Self learning material)

Pamphlets containing information with messages for men on safe motherhood, family planning, RTIs/STIs and HIV/AIDS were distributed to 2500 husbands in Mohili Village and 2500 couples in Bail Bazaar. Copies of 900 pamphlets providing information regarding Pap smear screening test and camps were distributed in each area. Copies of 4500 pamphlets providing information regarding clinic for men were distributed in Mohili Village and another 4500 pamphlets giving information regarding clinic for couples were distributed in Bail Bazaar along with the messages on safe motherhood, family planning, RTIs/STIs and HIV/AIDS.

Contact visits

Project team along with Community Health Volunteers (CHVs) made 102, 13 and 95, visits respectively to general physicians, Local mandal/NGOs and Volunteers in Mohili Village. The corresponding figures for Bail Bazaar are 109, 12 and 93 visits respectively. The purpose of the visit was to solicit General Physicians, Local

Mandal/NGOs and Volunteers co-operation in achieving the goal of the study.

Orientation programmes

Orientation programme on four issues (i.e., safe motherhood, family planning and contraception, RTIs/STIs and HIV/AIDS) of reproductive health was organized for medical and para-medical staff of Bail Bazaar health post. Nine staff members from Bail Bazaar attended for this programme.

Pap smear screening for Cervical Cancer

During April 2007 to March 2008 twelve camps each in Mohili Village and Bail Bazaar, were conducted. A total of 143 and 166 women from Mohili Village and Bail Bazaar attended and underwent Pap smear screening in these camps respectively. Pap smear findings indicate high prevalence of abnormal smears (Table 3 and 4).

Counselling clinic

As part of the intervention, counselling services were provided since September 2005 in the Mohili Village municipal health post and Bail Bazaar municipal health post. Counselling on problems such as white discharge, infertility, sexual problems, sexually transmitted infections, gynecological problems, burning micturition as well as information on reproductive health issues such as ANC/PNC, spacing contraceptive methods and Non Scalpel Vasectomy (NSV), were provided in these clinics once in a week on Monday at Mohili Village and Tuesday at Bail Bazaar health post. Suspected or HIV positive cases were also provided Counselling and referral. One hundred and forty husbands, 10 wives, 71 couples, four girls and a boy attended Counselling clinic at Mohili Village and 9 husbands, 155 wives, 23 couples, two boys and four girls attended to counselling clinic at Bail Bazaar. About 31 per cent husbands from Mohili Village and 11.9 per cent husbands from Bail Bazaar accompanied their wives for counselling.

Services for treating reproductive health problems

As a part of interventions, clinic for Reproductive Health Services, Clinic for Men was initiated at Mohili Village Municipal health post in the month of May 2006 and Clinic for Couples was initiated at Bail Bazaar Municipal health post for couples in the month of June 2006. Both the clinics are functioning on Sunday between 10.00 am to 1.00 pm. These activities were continued during the year 2007-2008. Problems regarding white discharge, primary and secondary infertility, sexual problems, sexually transmitted infections, gynecological problems, burning micturition, information on ANC/PNC, motivation for NSV and post NSV follow-up, counselling for HIV positive and suspected cases, motivation for condom use and information on reproductive health issues were provided in these clinics by medical and para-medical staff. About 70 per cent spouses from Mohili Village and 30.6 per cent spouses from Bail Bazaar accompanied their partner for reproductive health services.

The post-intervention survey started in July 2007 and was completed in December 2007. During this period 555 couples were interviewed from experimental area - 2 (Bail Bazaar), 598 from experimental area -1 (Mohili Village) and 575 from Control area (Asalfa Village). Coding and data entry has been completed for all three areas i.e. Bail Bazaar, Mohili Village and Asalfa Village. Data verification and data analysis is in progress.

Informal discussion with the beneficiaries reveals satisfactory results regarding intervention programmes conducted and services provided. Nevertheless, the change that has taken place in the overall knowledge, reproductive health attitude, inter-spouse communication, health seeking behaviour and the support provided by men to their spouses will be known after analysis of post-intervention data.

Table 3: Pap smear findings

Results	Mohili Village		Bail Bazaar		Total	
	Number	%	Number	%	Number	%
Negative	49	34.3	64	38.6	113	36.6
Inflammatory	84	58.7	95	57.2	179	57.9
Atypia	4	2.8	0	0.0	4	1.3
CIN I	2	1.4	3	1.8	5	1.6
CIN II	1	0.7	1	0.6	2	0.6
Carcinoma	0	0.0	1	0.6	1	0.3
Inadequate	0	2.1	10	1.2	5	1.6
Total	143	100	166	100	309	100
No. of Camps	12		12		24	

Table 4: Infections detected by Pap smear

Results	Mohili Village		Bail Bazaar		Total	
	Number	%	Number	%	Number	%
No infection	88	61.5	87	52.4	175	56.6
HPV	17	11.9	21	12.7	38	12.3
BV	16	11.2	19	11.4	35	11.3
MON	5	3.5	6	3.6	11	3.6
CHL	2	1.4	6	3.6	8	2.6
TV	1	0.7	2	1.2	3	1.0
Multiple infections	14	9.8	25	15.0	39	12.6
Total	143	100	166	100	309	100
No. of Camps	12		12		24	

(BV: Bacterial vaginosis, TV: Trichomonas vaginalis, MON: Moniliasis, CHL: Chlymidia)

Table 5: Clinic attendance (April 2007 – March 2008)

Indicator	Targeting Men (A)	Targeting Couples (B)	Total (A+B)
Men availing services	25 (12.7)	34 (7.0)	59 (8.6)
Accompanying partner to avail services	137 (69.6)	149 (30.6)	286 (41.8)
Women availing services	15 (7.6)	230 (47.2)	245 (35.8)
Boys availing services	2 (1.0)	19 (3.9)	21 (3.1)
Girls availing services	18 (9.1)	55 (11.3)	73 (10.7)
Total	197 [28.8]	487 [71.2]	684 [100.0]

Note: Figures in () indicates column percentage and figures in [] indicates row percentage.
Chi-square: 122.05, DF: 4, P<0.001