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RESEARCH IN INFERTILITY

It is recognised that infertility is multifactorial and several male and female factors may co-exist contributing to infertility in a given couple. It is therefore essential that an integrated approach is developed for management of infertile couples. Over the years the Institute for Research in Reproduction (IRR), Mumbai has evolved a detailed operational plan for evaluation and management of infertility with the main emphasis on male and female endocrine infertility and reproductive disorders.

The cause of infertility may vary from one geographic and social area to another. Globally the major causes of infertility in women are tubal occlusion and strictures, ovulatory disorders, sexually transmitted diseases and endometriosis. The causes of infertility in men are impaired spermatogenesis, disturbances in sperm-oocyte fusion, sexually transmitted diseases, agenesis of the epididymis and other parts of the ductal system including the vas deferens, anejaculation, *etc.* In the authors' experience male factors contribute to almost 50 per cent of infertility in infertile couples while the infertility in the remainders may be either due to a female factor or a combination of male and female factors. The factors frequently identified for infertility in patients at the IRR are shown in Table I.

Table I. Male and female factors identified for infertility in patients at the IRR, Mumbai

Male factors	Female factors
Idiopathic oligozoospermia	Non endocrine causes
Oligozoospermia due to varicocele	Anovulation without amenorrhoea
Oligozoospermia due to infection	Amenorrhoea with normal gonadotropins levels
Primary testicular failure	Amenorrhoea with increased gonadotropins levels
Obstructive azoospermia	Amenorrhoea with decreased gonadotropins levels
Idiopathic asthenozoospermia	Cervical infections
Asthenozoospermia due to infection	Hyperprolactinaemia
Anejaculation	Tubal factors
Hypospermia	Uterine factors
Abnormal sperm forms	Endometriosis
Hypothalamic pituitary failure	Corpus luteum dysfunction
Impotency	

In approximately 10-15 per cent of infertile couples no apparently cause can be found and such cases are categorized under Unexplained Infertility. Studies on female infertility were undertaken at IRR, Mumbai to (i) elucidate the pathophysiological mechanism in endocrine disorders *viz.* hyperandrogenaemia, hyperprolactinaemia and hypergonadism[1]; (ii) correlate clinical, morphological and

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histological features with endocrine profiles[2]; (iii) evaluate new drugs for induction or restoration of ovulation[3]; and (iv) evaluate alternate diagnostic tools[4,5]. The experience gained has enabled scientists at IRR to device algorithms for primary and secondary amenorrhoea, polycystic ovarian syndrome and recurrent abortions.

Reproductive Tract Infections

Reproductive tract infections, most of which are sexually transmitted diseases (STDs) can cause cervicitis, endometritis, pelvic inflammation, tubal sterility, spontaneous, early or recurrent abortion, prematurity, perinatal morbidity and mortality. The principal cause of inability to conceive is pelvic infection which occludes the fallopian tubes or leads to pelvic adhesions. Infection with *Neisseria gonorrhoea* or *Chlamydia trachomatis* obstructs the fallopian tubes. The relative contribution of each of these organisms to pelvic infection varies geographically and polymicrobial etiology is possible. These infections are more common in developing countries like India because of overcrowding, illiteracy, unhygienic conditions and poor diagnostic and treatment facilities. Chronic infections can lead to infertility, chronic pain and debility both in men and women.

Chlamydia trachomatis is to-day one of the commonest causes for STDs in the developing countries. Nearly half the infections are asymptomatic causing damage to the fallopian tubes leading to infertility and at times, life threatening conditions like an ectopic pregnancy. The prevalence of *C. trachomatis* infection and its relationship to abnormal laparoscopic findings relating to tubal damage are being studied in the infertile women attending the Infertility Clinic at IRR. Of the 100 women enrolled *C. trachomatis* infection was found in 30 per cent. A substantial number of *C. trachomatis* positive and negative patients were infected with other STD related organisms. The commonest amongst then was *Gardenerella vaginalis.* Abnormal laparoscopic findings were noted in 31 per cent of *C. trachomatis* positive patients. Although, different microorganisms contribute to infertility, the high prevalence of *C. trachomatis* infection and its relationship to pelvic inflammatory diseases suggest that in infertility management the routine screening for this organism is mandatory.

The spouse of the infertile women who has pelvic infection and total blockage of the fallopian tubes frequently presents serological evidence of prior chlamydial infection. Upto 35 per cent of men with partners having cervicitis due to *C.trachomatis*, are also positive for urethral chlamydial cultures and of these 86 per cent are asymptomatic. With increased societal permissiveness and tolerance and decline in the mean age at first sexual intercourse, particularly of male adolescents, increasing number of young men are contracting chlamydial urethritis which remains asymptomatic in a large number of cases. This group is probably a major source of chlamydial genital tract infection in adolescent or young adult female. Men with *C. trachomatis* infection do not show any alteration in sperm motility, morphology or count.

Evaluation of Probable Causes of Unexplained Infertility

Unexplained infertility *ie* a failure to conceive for more than 2 years with both the partners having no major or minor abnormalities, following basic investigations, was found in 10 per cent of the couples referred to the IRR's infertility clinic. A study was therefore undertaken to determine the probable causative factors of unexplained infertility (IRR Annual Report 1988-1989) *viz.* corpus luteum insufficiency (CLI), luteinized unruptured follicles (LUF), nidatory failure in women and sperm fertilizing capacity in men. In a recently completed study (IRR unpublished observations) 39 couples were identified as having unexplained infertility. Each female partner was studied for one to three menstrual cycles using clinical parameters such as basal body temperature, cervical mucus and endometrial biopsy; serum and urinary hormonal profiles and ultrasonographic scanning of ovaries for follicular development and rupture. Corpus luteum insufficiency was diagnosed if the urinary pregnandiol glucuronide levels were below 20th centile of the values in the active follicular phase. Serial follicular scans were utilised to detect LUF. Nidatory failure was determined by detection of early pregnancy factor (EPF) in the serum of female partners[6]. The male partners were evaluated for the fertilizing potential of their sperms by hamster egg penetration assay or by hypo-osmotic swelling test (HOS).

A total of 64 cycles were studied in the women. Corpus luteum insufficiency was diagnosed in 18 of the 64 cycles (28%) and in 13 of these it was due to abnormal folliculogenesis as determined by poor estrone glucuronide levels (< 20th centile of active follicular phase) and follicular size at ultrasound ($\leq$ 18 mm). In the remaining five increased preovulatory levels of prolactin were observed which could have interferred with folliculogenesis. Correction of folliculogenesis by clomiphene citrate resulted in one

pregnancy while normalisation of prolactin in the individuals with hyperprolactinaemia did not correct the CLI. The phenomenon of LUF was found to repeat itself in only one of two individuals in whom it was recognised. Early pregnancy factor was detected in six cycles. This suggested fertilization but, impaired implantation of the embryo probably due to defective endometrial development in spite of adequate circulatory progesterone levels. The observation of asynchrony of ovulation parameters in seven cycles suggested an abnormality of hypothalamo-pituitary function or a defect in the ovaries. Three cycles were found to be anovulated. No obvious cause was detected in the remaining 28 cycles. The incidence therefore of abnormal cycles in these "normal" infertile women was 51 per cent. Sperm fertilizing capacity was found to be subnormal in 9 of 29 males. However, in these individuals variability in some of seminal parameters *viz.* viscosity, motility was also noted.

In only 10 couples none of the above factors were identified and three of them conceived during the trial whereas following discontinuation of trials, three more conception occurred. To determine the frequency of successive normal or abnormal cycles 15 individuals were studied for three consecutive cycles. In five individuals all the three cycles were found to be normal. In two others only two of the three cycles were found to be normal while in the remaining eight all the cycles were abnormal. However, in five of them repeated CLI was observed. These observations point to the inherent variability in spontaneous cycles and also emphasise the need for an in depth evaluation of such couples, as real unexplained infertility was detectable only in 10 per cent of subjects.

In Vitro Fertilization and Embryo Transfer (IVF-ET)

In vitro fertilization of human eggs and implantation of cleaving embryos after transfer into the uterus to establish pregnancy had sparked a great deal of medical interest. The birth of Louise Brown in England in 1978 and the subsequent number of births in England, Australia and the United States, by this procedure had raised the hopes of many infertile couples in India. The IRR, Mumbai played a pivotal role in the advent of IVF-ET technology in India. Besides treatment of infertility in women whose tubes were blocked due to infection, another reason for developing this technique was to offer a method of achieving pregnancy in women who had undergone tubectomy and were desirous of having another child. With this in view, as early as in 1981-82 scientists at the IRR attempted collection of preovulatory oocytes by laparoscopy from women who were infertile due to tubal block and from those who had undergone tubal sterilization. These oocytes were used for establishing the *in vitro* fertilization technique.

Eighteen women aged below 35 years were initially recruited for the IVF-ET programme. Except for blocked tubes following sterilization there was no other cause for their current infertility. The husband's semen was evaluated by sperm penetration assay using zona free hamster eggs.

Since the number of embryos transferred increases the chances of pregnancy per patient, the women were administered clomiphene citrate 50mg/day or human menopausal gonadotropin (Pergonal) 150 I.U. per day or hFSH 1000 I.U. per day for multiple follicular stimulation and oocyte retrieval. Therapy commenced from day 3 in women where the cycle length was 28 $\pm$ 3 days and from day 5 when cycle length was 35 $\pm$ 3 days.

Follicular growth was monitored by cervical mucus scoring and/or ultrasound. When cervical scoring rose to 10/12 and/or ultrasound scans indicated follicles ranging from 17-20 mm, 5000 I.U. hCG was administered. Laparoscopic aspiration was done 36 hours later.

Out of the 26 follicles aspirated in 1983 at 14 aspirations in 12 women, only 3 preovulatory oocytes could be recovered. None of them fertilised *in vitro*. With change of therapy to hFSH, and monitoring with combination of clinical and hormonal parameters, 13 preovulatory oocytes could be obtained from 30 follicles aspirated at 13 aspirations in 12 women. Nine of these fertilized *in vitro* and 2 cleaved to 8 cell stage. Two embryo transfers were done but there was no pregnancy.

The IRR continued its sustained efforts to improve the IVF-ET programme and other assisted reproductive technologies. Various protocols for the induction of ovulation were attempted. Couples with male factor infertility and women with blocked tubes due to infection were also recruited.

Until 1990, 121 patients were treated for IVF-ET. Three protocols for ovarian stimulation were used and the results, in terms of number of oocytes available for retreival, fertilization rate, number of embryos transferred and pregnancy rates, were compared[7]. In the first protocol (n=3 cycles), the ovarian stimulating drugs (clomiphene citrate and human menopausal gonadotropin) were given according to a rigid dose regimen, the incidence of fertilization and pregnancy

were only about 10 per cent although the number of oocytes available for retrieval was maximal. In the second protocol (n=79 cycles) the dose regimen of the ovarian stimulating drugs was individualized on the basis of the length of the menstrual cycle; the results improved in terms of number of pregnancies (n=7) and live births (n=5). In the third protocol (n=30 cycles) where the day of initiating the ovarian stimulation was programmed by pretreating the women with oral contraceptives to suppress ovulation before starting ovarian stimulation therapy, the results in terms of fertilization rate were highest although the number of oocytes recovered was reduced. The techniques of IVF-ET, intra uterine insemination and oocyte donation for premature ovarian failure were successfully established resulting in 18 pregnancies by IVF-ET and 10 by gamete intrafallopian transfer (GIFT). Live births by IVF-ET were 10 and 4 by GIFT.

IVF-ET occupies a central position in assisted reproductive technologies (ARTs). Apart from the therapeutic value, the IVF-ET procedure has yieded valuable information about the responsiveness of the ovaries, normal and abnormal fertilization, pre-embryo score, implantation and pregnancy. ARTs beginning from simple intrauterine insemination combined with ovarian stimulation, gamete intrafallopian transfer, zygote intrafallopian transfer (ZIFT) have been used with variable success[8].

Micromanipulations have added a new dimension to IVF technology. Partial zona dissection (PZD), sub-zonal insemination (SUZI) and intra-cytoplasmic sperm injection (ICSI) have been used for improving fertilization. Men whose sperm do not achieve fertilization or whose sperm concentrations are too low have benefitted from SUZI where a few spermatozoa are introduced between the zona pellucida and the oolemma. ICSI involves the injection of a single spermatozoon directly into the cytoplasm of the oocyte. ICSI has been successful in patients with severe abnormal sperm morphology and asthenozoospermia with the use of spermatozoa retrieved from the epididymis. Trophoectoderm biopsy and blastomere sampling have been used for the purpose of pre-implantation diagnosis of genetic disorders.

Intrauterine Insemination in Management of Infertile Couples

Artificial insemination, either homologous (AIH) or donors has served an important role in the treatment of infertile couples. Indications for this include physiological and psychological dysfunction and abnormal semen characteristics. AIH has been performed by various techniques *viz.* intracervical, intravaginal or by using cervical caps. Of late, intrauterine insemination is being increasingly used for male subfertility or cervical mucus hostility before the couple is offered a more expensive and stressful programme of IVF-ET. The objective of such a regime is to deliver semen having high sperm density and improved sperm motility directly into the uterine cavity as close to the site of fertilization during or immediately after oocyte release when decreased sperm count, poor sperm motility, antisperm antibodies, cervical infection/hostility hinder conception. In order to assist couples with subfertility and provide higher chances for conception, 70 couples over a period of 4 years were subjected to intrauterine insemination (IUI) for a total of 120 cycles. Of these, 43 women had primary infertility and 27 had secondary infertility. In 53 of the 120 cycles, the chief indication for this procedure was poor cervical mucus with poor sperm motility in males. Majority of cycles (80%) were anovulatory and ovulation was induced with clomiphene citrate, human menopausal gonadotropin or a combination of both. Eighteen of the 70 couples conceived giving a pregnancy rate of 25.5 per cent per couple and 15 per cent per cycle. These results suggest that IUI with controlled ovarian stimulation can serve as a useful assisted reproductive procedure in individuals who have resistant factors for infertility before offering them other expensive ARTs.

Human Semenology

Semen analysis has generally been utilized in the evaluation of male fertility. In order to establish the reference norms for semen analysis in the infertility clinic at the IRR, Mumbai, studies were undertaken for comparison of semen characteristics in infertile couples with that of males with recently proven fertility. The details of periconceptional semen analysis in men of proven fertility and husbands of infertile couples whose wives became pregnant while under therapy, were obtained. In the former group semen analysis was carried out within 6 to 36 weeks of pregnancy. The volunteers were husbands of women who attended the antenatal clinic or requested for medical termination of pregnancy. In this group all the couples had proved fertility prior to the current pregnancy. In the infertile group, semen analysis was done during the 3 months periconceptional period.

The values for sperm count, total motility as well as active forward progressive motility in this analysis showed much lower values than normal fertile and in males where no male factors could be detected. In 1979 the IRR laboratory norm was a count of 20 million/ml. However, 14 of 105

subjects impregnated successfully with a count less than 20 million/ml. Moreover the laboratory norms for motility were such that spermatozoa should show 30 per cent active forward progressive motility. In this series of periconceptional semen analysis, 60 of 105 subjects with less than 30 per cent active forward motility impregnated successfully. It was felt that for reference norms for semen analysis, a very large series of representative, properly selected subjects must be studied.

As a follow up of this study semen samples from fertile individuals were continued to be analysed. Seminal fluid smears were prepared, fixed and stained with Papanicolaou stain, and semen morphology evaluated.

Efficiency of routine semen analysis to predict functional and structural integrity of human spermatozoa

Routine analysis[9] : The physical characteristics studied were volume, liquefaction time, viscosity and pH according to WHO Manual. Morphology was scored on smears stained with Papanicolaou stain.

Scoring system : From the multitude of parameters studied, 10 were selected and a scoring system was applied. For the purpose of scoring, semen samples were separated into 3 groups *viz.* fertile, subfertile and infertile, on the basis of previously standardised normative values (Table II). Each of these group was assigned an arbitrary score of 2, 1 and 0 respectively. Thus, any given sample could have a maximum score of 20 (fertile) and minimum score of zero (infertile). The distribution of scores for the routine tests in the fertile, subfertile and infertile groups was 20-16, 15-11 and 10-1 respectively, while that for the functional tests was 10, 9-5 and 4-0 respectively.

Functional analysis : Functional tests were done to measure the dynamic capability of spermatozoa. Each test was directed against a specific organelle of spermatozoa.

(i) Hypoosmotic swelling test (HOS test) : This test was done to check the integrity of sperm plasma membranes and was carried out according to the method of Jeyendran *et al*[10].

(ii) Test for acrosome intactness (TAI)[11] : TAI was done using a gelatin slide. The test is based on the principle that acrosomal protease hydrolyses the high molecular weight protein like gelatin.

(iii) Nuclear chromatin decondensation (NCD)[12] : This was done to check the compactness and the ability of spermatozoa to decondense *in vitro*.

(iv) Sperm mitochondrial activity index (SMAI)[13] : SMAI is a quantitative cytochemical test to determine the mitochondrial activity and thereby motility of spermatozoa.

Table II. Categorization of values of routine parameters in semen samples from 3 groups of subjects.

Parameter	Groups		
	Fertile	Subfertile	Infertile
I. Routine			
Liquefaction time (min.)	20-34	34-45	> 4.5
Volume (ml)	1.5-4.5	4.6-5.0	> 5.0
		1.2-1.4	> 1.2
Viscosity	Normal	Moderate	High
Particulate matter	Nil-Mild	Moderate	Severe
Agglutination (%)	Nil	1-10	> 10
Motility (% RLP)	> 25	10-24	< 10
Vitality (%)	> 60	40-59	< 40
Density (mil/ml)	> 20	10-19	< 10
Morphology			
Normal forms (%)	> 30	20-29	< 20
Headless spermatozoa	> 20	20-25	> 25
II. Functional			
HOS (%)	> 60	50-59	0.49
Halo (%)	> 50	40-49	0-39
Halo diameter (μm)	> 35	20-29	0.19
NCD (%)	> 70	60-69	0-59
SMAI	> 50	40-49	0.39

RLP - Rapid linear progressive
HOS - Hypoosmotic swelling
NCD - Nuclear chromatin decondensation
SMAI - Sperm mitochondrial activity index

Thus the 5 parameters in functional analysis were (i) HOS, TAI (ii) the halo percentage, (iii) halo diameter, (iv) NCD and, (v) SMAI and the maximum score for functional analysis was 10. The scoring system was uniform for both routine and functional analysis. The group under which each sample was categorized for routine and functional analysis was then compared.

Incidence of headless spermatozoa in the semen of fertile and infertile men

Spermatozoa exhibit certain forms of morphological abnormality commonly referred to as 'Pinheads'. These were found to be actually 'headless' sperms as determined by phase contrast, light and electron microscopy. This morphological defect was studied in the semen samples assessed for routine characteristics. These sperms have only a midpiece and tail. As these sperms do not have any of the organelles essential for oocyte penetration and fertilization,

a correlation was sought between the incidence of 'headless' sperms and infertility in men. A significant increase in the incidence of headless spermatozoa ranging from 10 to 43 per cent was found in semen samples obtained from infertile men. Hence the per cent incidence of headless spermatozoa could be used as a crude parameter to evaluate the quality of semen samples. It provides a possible mechanism for infertility since headless spermatozoa have virtually no fertilizing potential[14].

Role of bacteriological study in selection of cases for IVF and embryo transfer

The influence of bacteria on sperm characteristics specially in asymptomatic males is not known. It is important that the role of seminal bacteria in infertility and subfertility be understood specially with the use of techniques like IVF and GIFT. Bacteriological investigations were carried out on 50 semen samples from asymptomatic men to be enrolled for the IVF-ET programme. Seminal parameters were correlated with bacteriological findings. Samples were cultured for both anaerobic and aerobic bacteria. Most semen samples (n=44, 88%) contained two or more bacterial species, the rest (n=6, 12%) did not show any bacterial growth. The presence of bacteria in colony counts of 10^2/ml was correlated with seminal characteristics. A colony count of $>10^2$/ml was considered positive. A significant reduction in count and motility was observed in all positive specimens.

Amorphous particulate matter as seen by wet smear was moderate (+++) to severe (++++) in all bacteriologically positive cases. The pH ranged between 7.2-8.1 and showed no difference between the positive and negative cases. Viscosity measurements showed high or abnormal viscosity to be associated with bacteriologically positive cases and no case with abnormal viscosity had negative culture. Majority of the cases (81%) with normal viscosity had negative bacterial culture. Percentage of abnormal morphology was increased in positive cases (88%) along with abnormal HOS test results.

The observation of amorphous particulate matter was found to be a good indicator of culture positivity or negativity. The percentage of pus cells had no correlation with the occurrence of subclinical infection. The results of the HOS-test indicates that bacterial infection might possibly alter sperm membrane integrity and thereby the fertilizing ability. This study suggests that sperm characteristics are severely altered in asymptomatic men with bacterial infection. The presence of a bacterial colony count of $> 10^2$/ml in the semen sample, irrespective of whether it is a contamination from urethera, skin or environment, is of importance. It also establishes a causal relationship between bacteriospermia and subfertility. Further, the results of this study highlight the need for carrying out bacteriological studies even in asymptomatic men prior to enrollment for IVF-ET[15].

Seminal viscosity as a predictor of fertility status of the sample

As viscosity of the seminal fluid is one of the important parameters to determine the quality of semen, this was studied in detail. A new needle and syringe method was developed which was validated with other viscometers. This method is useful in quantitating viscosity. The seminal viscosity readings were correlated with other routine parameters like count, motility, morphology, particulate matter, *etc.* A total of 208 cases were studied. There was no correlation of seminal viscosity with sperm count. Normal viscosity was accompanied by normal motility and viability whereas abnormal viscosity was partly associated with abnormal motility and viability. Samples having high or abnormal viscosity had high percentage of particulate debris (86.6%) and high percentage of morphologically abnormal forms (96.5%). In general increased viscosity was associated with a high per cent particulate matter; increased per cent of morphologically abnormal forms; positive bacterial culture; presence of sperm antibodies; increased occurrence of amorphous particulate matter; and lower per cent motility and viability.

Hence quantitation of seminal viscosity is a useful predictor of the quality of the sample.

Assessment of mitochondrial activity of human spermatozoa with reference to motility and viability in fertile and infertile men

Since motility is an important parameter to judge the quality of seminal fluid, it was considered worthwhile to develop a quantitative method of judging motility as compared to routine subjective method.

The mitochondrial activity of human spermatozoa was evaluated by a cytochemical method using nitroblue tetrazolium salt[13]. The objective behind developing the test was to quantitatively assess the mitochondrial activity and to check whether there exists any correlation with the percentage of motile spermatozoa in the sample.

A significant decrease in the mitochondrial activity was observed in the infertile group. There was a significant correlation between motility and mitochondrial activity. There was no significant association between mitochondrial activity and sperm viability. This is thus a simple, quantitative, objective approach to judge the overall change in sperm motility in fresh semen and is therefore of diagnostic value and can be used as an indicator in various experimental conditions.

Sperm function in asthenozoospermia

With the understanding of routine semen analysis and development of sperm function tests, a structure and function study on severe asthenozoospermia was undertaken[11]. The study was done on 25 patients with severe sperm motility problems. The parameters included routine analysis, functional tests, ultrastructural study and scoring system. In this study, eventhough Kartegener's syndrome was suspected in 5 subjects (with history of recurrent cold, cough and bronchitis) dynein arms of the flagella were absent in only one case and that too not as an isolated defect. Both inner and outer dynein arms were absent. Ultrastructural study revealed multiplicity of defects. Mitochondrial defect may be one of the cause for the loss of sperm motility in this population.

Factors Contributing to Repeated Pregnancy Wastage

Recurrent spontaneous abortion is a phenomenon of varied etiology *viz.* genetic, immunological, endocrinological, uterine anomalies and subclinical infection. The cytotoxic effects of infectious agents and the resultant inflammatory responses may interfere with organogenesis or histogenesis in the foetus. In an attempt to assign either a high or low risk for subsequent pregnancy losses, characteristics of etiological factors such as uterine anomalies, endocrine dysfunction and subclinical infections in women were studied. The interim results indicate that a significant number of women are exposed to the risk of infectious agents. The infections identified in the order of their frequency are toxoplasmosis (32%), cytomegalovirus (20%), rubella (16%), *Chlamydia trachomatis* (16%) and syphilis (8%). Uterine anomalies such as submucus polyp or incompetent os were seen in 14.2 per cent. Corpus luteum inadequacy was observed in 3 women only. These observations suggest that increasing exposure of women to microbes leads to chronic maternal genital infections.

Semen samples from males with partners having bad obstetric history (BOH) have also been studied, so as to identify the seminal characteristics and the functional capacity of spermatozoa in this group of men. The results indicate increase in sperm head abnormalities along with nuclear chromatin abnormalities. The study shows that apart from a number of possible causes of BOH in the female *viz.*, genetic, immunologic, endocrine, infection and uterine organic factors; contribution from the male could be considered as one of the possible etiological factors. Hence, a comprehensive evaluation of the male spouse is indicated.

Cytogenetic Abnormalities as a Cause of Male Infertility

Chromosomal abnormalities have been known to be one of the important causes of infertility. The frequency of chromosomal aberrations has ranged from 1-3 per cent amongst all infertile men. However, when karyotyping was restricted to azoospermic men, this incidence rose to 15 per cent. In several cytogenetic surveys in oligoazoospermic males, the incidence ranged from 6-8 per cent.

Oligoazoospermic males (n=51), where infection and surgical causes were excluded were karyotyped[16]. Of these, six individuals had abnormal karyotype, giving an incidence of 12 per cent. Three of them had 47, and one had 46, XY/47, XXY mosaic Klinefelter syndrome. One had a deletion of long arm of Y and one had a mosaicism for 46, XY/47, XYY.

Significance of minor cytogenetic abnormalities in male infertility

The use of Giemsa banding technique for karyotyping has revealed several minor abnormalities in individuals with male infertility. These are abnormal length of Y chromosomes and enlarged satelites - acrocentric chromosomes (13-15 and 21-22). Family studies in these cases have been informative. Of the three cases where an enlarged Y was found, paternal chromosomes could be studied in two. Both these fathers had a long Y similar to the patients. These men were normally fertile. It is felt that a large Y chromosome may not compromise the reproductive function of an individual. A short or a deleted Y chromosome, however, is more likely to be the causal factor in male infertility. One patient who had azoospermia was found to have Yq- with an absence of the fluorescent part of the Y chromosome. This indicates that the fluorescent part of Y may be crucial for proper spermatogenesis. Recent reports also indicate

microdeletions in the Y chromosome to be a cause of male infertility[17].

Two large comprehensive studies in male (oligozoospermia, 102 cases) and female (primary amenorrhoea, 123 cases) reproductive disorders at IRR defined the selective population for which cytogenetic studies can be applied. The study showed that though the occasional patient with cytogenetic abnormality may have normal gonadotropins, most often abnormality was detected in those who had elevated serum FSH. It was clear that a study to determine the frequency of cytogenetic abnormalities in male and female reproductive disorders can be restricted only to the latter group. The study also provided interesting observations like high frequency of isochromosomes for X in the primary amenorrhoea group and rare abnormalities such as familial metacentric Y and XY/XXY in persistent mullerian structure syndrome in males.

Analysis of spermatozoa chromosomes

Analysis of human sperm chromosomes was made possible by activating sperm within zona free hamster eggs. This technique is of immense value in evaluating the normality of sperm chromosomes. The technique has been standardized. At present, analysis of sperm chromosomes from male partners of couples having a history of spontaneous abortions is being carried out.

Conclusion

Most of the studies have been done to gain an insight into the causes of infertility more so of male infertility. The basis of the studies on male factor infertility is to focus and standardise simplified techniques and options of infertility treatment and to make available to most people. Our data give value to the first line of therapy such as intrauterine inseminations in male factor subfertility before attempting the use of sophisticated techniques such as IVF. In the absence of an adequately supported ART programme these simpler procedures should be developed for the economically poorer sections of the population. This will require special emphasis on intracytoplasmic sperm injection which will apart from offering treatment, provide good research material. Infections like STDs present as the most important and most preventable cause of infertility hence these should be studied in detail. Apart from the purely economic reasons one more reason for concern is the prevalence of multifactorial infertility. The diagnosis and treatment/management of infertility being a multifactorial problem requires a lot of basic understanding of the endocrine, genetic, psychological aspects of both the partners.

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This write-up was contributed by Dr. Kamala Gopalkrishnan, Dr.Pervin K. Meherji and Dr. H.S. Juneja, Institute for Research in Reproduction, Mumbai.

ABSTRACTS

Some Research Projects Completed Recently

Association of *(Campylobacter pyloridis) Helicobacter pylori* in patients undergoing gastroscopy for benign peptic and duodenal ulcers

Antral mucosal biopsy tissues of 100 patients each of non-ulcers dyspepsia, benign gastric ulcer and with other diseases of gastrointestinal tract were studied for analysing the association of *Helicobacter pylori* with disease. The biopsy samples obtained on endoscopy from apparently healthy region of the antrum away from the ulcer site served as control. The biopsy samples were subjected to urease tests (indigenously standardised), culture and histopathology.

H. pylori was found to be significantly associated in patients with gastritis and gastric, duodenal ulcers and gastric carcinoma. Brain heart infusion agar with 7 per cent horse serum, growth supplements and Butzler antibiotic supplements supported the growth of *H. pylori*. SDS-PAGE analysis revealed the presence of all the major proteins except the 120 kDa protein which was not present in all the isolates. A liquid and a semi-solid urease test standardized indigenously was found to be comparable and cost effective than the commercially available CLO (Campylobactor Like Organism) test.

Cytopathic effect was produced by broth-culture filterates of *H. pylori* confirming the production of a cytotoxin in HeLa cell line. Intraperitoneal injections of cell sonicates or live suspensions of *H. pylori* produced lethality in Balb/c mice. However injections of heat treated suspension did not produce such effects confirming the production of heat labile toxin by *H. pylori*.

Analysis of data on clinical history of patients failed to reveal the role of alcohol and smoking as associated risk factors in the ulceration process in the patients with *H. pylori* infection. Irregular dietary habits seemed to play a role in the ulceration process.

PCR analysis showed the presence of similar DNA sequences in both *Campylobacter jejuni* and *H.pylori*.

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Calcium homeostasis in isolated human parietal cells in normal and altered physiological states

The study was carried out to ascertain the basal status of intracellular free calcium and calcium transport across the plasma membrane in an isolated parietal cell population derived from patients with and without duodenal ulcer.

Multiple endoscopic gastric mucosal biopsies from the corpus of the stomach in patients undergoing endoscopic evaluation for epigastric pain/reevaluation of duodenal ulcer were processed and isolated parietal cells were studied. Gastric biopsy tissue taken 5 cm away from the lesion in subjects with suspected gastric carcinoma but having normal acid levels were used as controls. Patients taking calcium antagonists and those concurrently taking H_2 antagonists were excluded. Relevant clinical details including

smoking habits, alcohol intake, and drug history were recorded. Intracellular calcium was estimated in 21 patients using fura-2-acetoxymethyl ester. Calcium influx was determined in 26 patients and efflux in 12 patients (in 10 patients both influx and efflux could be performed together) by using radioactive calcium. Acridine orange retention was used to assess acid production by parietal cells in 20 patients.

A homogeneous population of viable (>75%) parietal cells was obtained with a yield of 1.75×10^6 cells/ml. The cells had characteristic eosinophilic cytoplasm and concentric nucleus. The purity of the preparation was demonstrated by a modest three-fold rise in K^+- dependent paranitrophenyl phosphate activity from the "crude" to the pure after precoll density gardient. Patients with an active duodenal ulcer had a higher intracellular free calcium (239.75 nmol/10^6 cells) as compared to patients who had nonspecific endoscopic findings (mean 143.36 nmol/10^6 cells) and patients with normal endoscopy (mean 145.6 nmol/10^6 cells). The acridine orange retention as a measure of acid secretion was higher but comparable in patients with active duodenal ulcer (32.55%) and nonspecific endoscopic findings (35.32%) as compared to individuals with normal endoscopy.

Only calcium influx at 20 min was significantly more in patients with duodenal ulcers as compared to the control group and those with nonspecific changes at endoscopy. There was no difference between the groups (with active duodenal ulcer, non-specific changes at endoscopy and normal endoscopy) in calcium influx at 0 and 60 min; calcium efflux at 0, 20 and 60 min. None of the parameters tested showed consistent variations that could be attributed to smoking, alcohol intake or presence of nonspecific changes at endoscopy.

It is thus concluded that in the unstimulated state calcium homeostasis in isolated parietal cells of patients with duodenal ulcer shows only a minimal difference as compared to controls.

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Publication

Kaur, U. Agnihotri, N., Kaur, S., Khuller, M., Bambery, P., Sood, G. and Singh, K. Duodenal ulcer: Calcium status in isolated parietal cells. *Dig Dis Sci 40:* 887, 1995.

ICMR NEWS

Shri H.D. Deve Gowda, Hon'ble Prime Minister of India,presented the ICMR Awards and Prizes to outstanding Biomedical Scientists on October 15, 1996. He also launched the concluding ceremony of the year long 85th Anniversary celebrations of the ICMR. Shri Saleem I. Shervani, Hon'ble Minister of State for Health and Family Welfare and President ICMR, presided over the function.

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The following meetings of various technical committees/groups of the Council were held:

Meetings of the Scientific Advisory Committees of the ICMR Institutes/Centres:

Institute of Cytology and Preventive Oncology, New Delhi.	September 25, 1996
Institute of Immuno-haematolgy, Mumbai	September 26, 1996
Tuberculosis Research Centre, Madras	September 27, 1996
Institute for Research in Medical Statistics (both Delhi and Madras chapters) held at Madras	September 28, 1996
National Institute of Nutrition, Hyderabad	October 4, 1996
Food and Drug Toxicology Research Centre, Hyderabad and National Centre for Laboratory Animal Sciences, Hyderabad	(October 5, 1996)

Meetings of the Scientific Advisory Groups (SAGs)/ Scientific Review Committees (SRCs)/ Project Review Committees (PRCs)/Working Groups/Task Forces (TFs) held at New Delhi:

SRC of the Division of Reproductive Health and Nutrition	September 17-18, 1996

Working Group of the National Committee for Research on Human Reproduction (NCRHR) Sub-committee on Socio-behavioural Aspects of Reproductive Health Research	September 19-20, 1996
Working Group of NCRHR Sub-committee on Basic and Applied Research in Human Reproduction	September 25-26, 1996
PRC for Projects in Non-Communicable Diseases	September 27, 1996
SAG of the Division of Publication and Information	September 30, 1996
Working Group of NCRHR Sub-committee on Clinical Research in Human Reproduction	September 30 – October 1, 1996
TF on Monitoring of Adverse Drug Reactions in India	October 8, 1996
Working Group of NCRHR Sub-committee on Operational Research in Human Reproduction	October 11-12, 1996

Participation of ICMR Scientists in Scientific Events:

Dr. P.K. Nag, Asstt. Director, National Institute of Occupational Health, Ahmedabad, participated in the International Congress on Occupational Health '96 at Stockholm (September 15-20, 1996).

Dr. D.K. Das, Officer-in-Charge, Institute of Cytology and Preventive Oncology, New Delhi, participated in the XXIV European Congress of Cytology at Ljubljana (September 21-24, 1996).

Dr. T. Shanta Devi, Dy. Director, Tuberculosis Research Centre (TRC), Madras, participated in the meeting of the International Union Against Tuberculosis and Lung Diseases (IUATLD) at Paris (October 2-5, 1996).

Dr. C.N. Paramasivan, Dy. Director (Senior Grade), TRC, Madras, participated in a joint WHO/IUATLD Global Working Group Workshop on Antituberculosis Drug Resistance Surveillance at Paris (October 6-7, 1966).

Dr. S.K. Bhattacharya, Director, National Institute of Cholera and Enteric Diseases, Calcutta, delivered the Alastair Ironside Memorial Lecture of 1996 at Manchester (October 10, 1996).

Dr. M.D. Gupte, Officer-in-Charge, CJIL Field Unit, Avadi, Madras, participated in the meeting of the WHO Task Force on the Monitoring and Evaluation of Elimination of Leprosy, as also in the II International Conference on the Elimination of Leprosy as a Public Health Problem at New Delhi (October 10 and 11-13, 1996 respectively).

Oration:

Prof. V. Ramalingaswami, National Research Professor and Professor Emeritus, All India Institute of Medical Sciences, New Delhi, delivered the II Dr. B.R. Ambedkar Centenary Award (1993-94) Oration of the ICMR on "Changing Paradigms of Infectious Diseases in Developing Countries" at Vigyan Bhavan, New Delhi on October 15, 1996.

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The Council announces with deep regret the sad demise of three of its scientists *viz* Dr. V. Dhanda, Director, Vector Control Research Centre, Pondicherry, on August 20, 1996; Dr. P. Chattopadhyaya, Asstt. Director, National Institute of Occupational Health, Ahmedabad on August 16, 1996; and Dr. D.S. Chaudhury, formerly Deputy Director of the Malaria Research Centre, Delhi on June 6, 1996.

ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

Symposium/Seminar/Workshop/ Course/Conference	Date & Place	Contact Address
XX National Congress of Indian Association of Medical Microbiologists.	October 31 - November 3, 1996; (at Agra)	Dr. V.M. Katoch, Organising Secretary of the Congress, Department of Microbiology, Central Jalma Institute for Leprosy, Agra-282001.
Symposium on Arid Ecosystem of Thar Desert of Rajasthan and Evolutionary Ecology and Logic of Life.	November 1-3, 1996; (at Jodhpur)	Dr. A.R. Reddy, Convenor of the Symposium, Defence Laboratory, Jodhpur-342011.
International Workshop on Prospects of Medicinal Plants.	November 4-9, 1996; (at Nauni-Solan)	Dr. P.L. Gautam, Organising Secretary, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan-173230.
Second Asian and Oceanic Congress of Andrology.	November 16-20, 1996; (at Chandigarh)	Dr. N.R. Kalla, Chairman of Organising Committee of the Congress, Department of Biophysics, Panjab University, Chandigarh-160014.
XX National Conference of the Indian Society for Surgery of Hand.	December 7-8, 1996; (at Agra)	Dr. S. Husain, Organising Secretary of the Conference, Department of Plastic and Reconstructive Surgery, Central Jalma Institute for Leprosy, Agra-282001.

COUNCIL'S TRAINING PROGRAMMES

Nutrition

At the National Institute of Nutrition, Hyderabad:

Annual Training Course in Nutrition (December 1, 1996-February 28, 1997).

Laboratory Animal Technology

At the National Centre for Laboratory Animal Science, National Institute of Nutrition, Hyderabad:

Training Course for Laboratory Animal Supervisors (September 2–November 29, 1996).

Haematology

At the Institute of Immunohaematology, Mumbai:

Training Course in Advanced Haematology and Immunohaematology (September 17–October 4, 1996).

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