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# ICMR BULLETIN

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## DECREASING SPERM COUNTS – FACT OR FICTION

Recent years have seen numerous reports in the publication of a variety of specialities including medicine, chemical engineering and environmental sciences regarding a precipitous drop in sperm quality. These reports have also been given extensive coverage in the lay press fanning the fires of a full scale controversy. However, the retrospective nature of the data, varying methods of analysis and lack of quality control have all contributed to the truth not being clear. Given the sensitive nature of the issue it has become mandatory to understand whether this phenomenon is fact or fiction.

### Historical Aspects

The issue was raised in 1992 by the controversial study of Carlsen *et al.*[1] who performed an extensive linear regression analysis on 61 different sets of data between 1938 - 1990, reporting a decrease in mean sperm concentration of more than 40 per cent (from 113 x $10^6$/ml to 66 x $10^6$/ml). This was following McCleod's[2] 10 year study in USA published in 1979 which had reported no such change. Carlsen's paper[1] received extensive coverage in both, the international press and audio visual media and the issue captured popular imagination. This paper was followed by numerous papers both for and against this thesis. Emphasis was placed on not only declining sperm count[1,3-9] but also in decrease in seminal volume[1] and increase in abnormal forms[8,10]. These data were used to extrapolate regarding the fertility status of the generation after the late nineteen fifties.

### Trends Over Time and Place

Available reports were predominantly from European (England, France, Denmark, Scotland and Finland) countries[6, 11-15]. In addition, reports about geographical variations within countries and regions[16,17] were published. During the same period several reports appeared which denied any such decline[13,18]. Others in the field without quoting actual data, criticised the "pro-decrease" papers on statistical grounds[19] and criticised the use of the linear regression model. They recommended that the quadratic and spline-fit models would fit the data better. These models show either no change or even a slight increase in the sperm count. The studies were also criticised over methodological issues such as varying methodology for semen analysis, poor quality control, variation in sexual abstinence, varying methods of semen collection, all of which prove confounding[5,11,20]. Others have attributed this change to selection bias with the variability of study group with respect to geography, race and seeming "normality"[16,19,21-24]. Further statistical problems in the form of lower reference values[11] use of mean and not median[5,11]. Doubts were also raised for the validity and reliability of the early data[19,25]. Various studies have reported a decline in various aspects of male reproductive function. These

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include decrease in sperm counts[3-9], decrease in seminal volume[1], increase in abnormal forms[4,10,12] and decrease in fertility[4,12].

## Etiological Theories

Different theories have been put forward as a possible explanation of this phenomenon. Some authorities have reported an increase in testicular cancers in different industrialised countries concurrent with decrease in sperm quality. There was a birth cohort pattern observed for data after the mid fifties. This is attributed to etiological factors of testicular cancer by 3-4 times, operating early in life probably in utero[26-30]. Secular trend in the incidence of testicular cancer in selected areas is given in Table I[31]. Simultaneous reports of an increase in the

**Table I: Secular trend in the incidence of testicular cancer in selected areas (annual rate per 100 000)[31]**

| Country | 1960-63 | 1964-68 | 1969-71 | 1973-77 | 1978-82 | 1983-87 |
|---|---|---|---|---|---|---|
| Denmark | 3.8 | 4.5 | 4.9 | 6.7 | 7.8 | 8.4 |
| Finland | 1.2 | 0.8 | 1.1 | 1.7 | 1.5 | 1.8 |
| UK (South Western Region) | 2.1 | 2.4 | 2.6 | - | 3.8 | 5.0 |
| Germany (Hamburg) | 2.6 | 4.0 | 4.7 | 5.4 | 5.7 | - |
| USA (Los Angeles, white men) | - | - | - | 3.6 | 4.7 | 5.8 |
| Puerto Rico | 0.3 | 0.7 | - | 0.5 | 1.0 | 1.2 |
| Japan (Miyagi) | 0.6 | 0.4 | 0.8 | 0.8 | 1.0 | 1.3 |

Superscript number is the serial no. in the reference list.

incidence of cryptorchidism and hypospadias with decreased semen volumes and sperm count were reported in the sons of women treated with synthetic estrogen diethylstilbestrol (DES) between 1945 and 1971[32,33]. The prevalence of hypospadias at birth is given in Table II[34].

**Table II: Prevalence of hypospadias at birth (rate per 10000; World Health Organization, 1991)[34]**

| Country | 1970 | 1980 | 1990 |
|---|---|---|---|
| England and Wales | 9.5 | 15 | 12 |
| Hungary | 6 | 22 | 17 |
| Sweden | 16 | 20 | 18 |
| Norway | 5 | 15 | 17.5 |
| Denmark | 8 | 10 | 14 |
| Finland | - | 4 | 5 |

Superscript number is the serial no. in the reference list.

Reduced fertility was also attributed to foetal Sertoli cell damage[35]. Underlying all these varying factors was an increased estrogenic influence. The final common pathway of estrogenic influence led investigators to postulate whether the fall in sperm count could be attributed to a change in estrogenic influences over the last half century (Fig.1). The sources of these estrogens have been

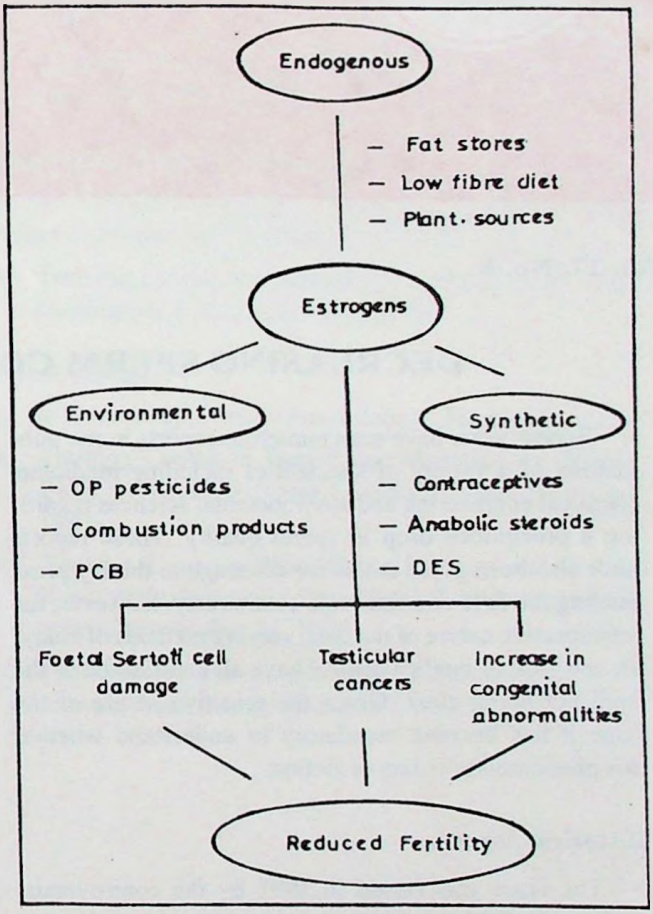



**Fig. 1. Sources of estrogens and their effect on fertility**
OP -Organophosphorous; PCB Polychorinated biphenyls;
DES - Diethyl stilbestrol

postulated to be both endogenous and exogenous. Endogenous increases could have resulted from changing diets which increase the enterohepatic circulation of estrogens and increase in body fat may also have increased the bioavailable estrogens by converting other steroids into estrogens. Synthetic estrogens may have increased through use of oral contraceptives by women and orally active anabolic steroids in the live-stock. Increased consumption of dairy produce and food substances containing soya may account for hyper estrogenaemia[36] with changing dietary fads and fashions. In addition to which

many such estrogenic chemicals such as organochlorine compounds and combustion products present in the food chain further add to the body store of estrogen. Some organo compounds like dichloro diphenyl trichloro ethane and polychlorinated biphenyls (PCBs) are remarkably resistant to biodegradation[37,38]. These factors total upto influences from occupation, environment, diet, life style and contraception. Various factors like stress, smoking, sexually transmitted diseases and food habbits make this problem more complicated. Sharpe and Skakkebaek[37] report that humans now live in an environment which is a virtual sea of estrogens.

**What Needs to be Done**

This phenomenon of decline in semen quality suffers from an incomplete description of its essential aspects in spite of a surfiet of publicity. There are numerous questions which have been partly answered and others not at all.

The epidemiological aspect of this decreasing trend in sperm counts has to be studied world-wide. Even within various centres there are reports of geographical variations[16,17] within regions. The implications of these geographical variations with regard to etiology are worth exploring. Some workers have attributed these variations to environmental nutrition and other causes[16]. Others have attributed it to difference in protocol of analysis and yet others have stated that these differences may be inherent. The differences taking into account the race, ethnic background and life style (socio-economic) have not been clearly done. This forms an important area for research in fertility. There is a paucity of material from the third world countries which needs to be addressed. There is also lack of prospective data on larger populations which would be most predictive of definitive trends. Prospective studies will also address the lacunae in methodology which could invalidate previous studies. These would include use of standardised analytical methods, unbiased selection accounting for differences in age, race, socio-economic status, urbanization, ethnicity, medical and reproductive history, life-style patterns and occupation. There is a need to evolve stringent quality control with rigorous record keeping. Prospective studies using predecided reference values and employing means of proving fertility will greatly add to the reliability and validity of the data. Possible environmental influences ranging from environmental factors to dietary changes will have to be explored.

Most of the etiologies regarding this decline in sperm counts have remained at the stage of hypothesis. There is a need to verify numerous postulates. This will include pharmacokinetic studies in xenobiotics, study of the effects of various synthetic estrogens like hormonal contraceptives on human and use of anabolic steroids on the live-stock. Study of dietary estrogenic influences like obesity, low fibre diet and physoestrogens on fertility should be undertaken. This will also include a thorough review of veterinary medicine for the effect of global environmental factors on animal fertility. The possible contribution of organophosphorous and organochloride insecticides and pesticides, PCBs and combustion products on impairment of fertility needs urgent investigation.

**The Indian Scenario**

The Institute for Research in Reproduction (IRR), Mumbai, has been working in the field of semenology and human sperm quality for over 15 years. Data on semen parameters of more than 1500 men from various parts of the country over the last 10 years are available. These data represent both normal populations and selected population attending the Infertility Clinic at IRR. The Institute is in a unique position of employing the same staff and equipment over the entire period of the study. The study used standardised methodolgy and employed stringent quality control. The preliminary analysis of the data indicated a definitive trend of decline in semen quality. There is a decrease in the mean percentage of normal morphology with less than 30 per cent normal forms (Fig.2). This corroborates the findings of Mehta and Anand Kumar[39] who have reported a similar decline in a study of population from Bangalore, India. This decline in the study from Bangalore is correlated with changes in various air pollution indices like suspended particulate matter, sulphur dioxide and lead.

**Animal Studies**

The reports of decline in semen quality are not only limited to humans. Changes in male reproduction and reproductive capacity in wild life and avian populations have been linked in many cases with environmental pollutants. Experimental evidence shows that environmental pollutants disturb reproductive function in rhesus monkeys, hens and in fish eating birds of North America and United Kingdom[40]. A compound called tributyrin leaching from the marine antifouling paints has been

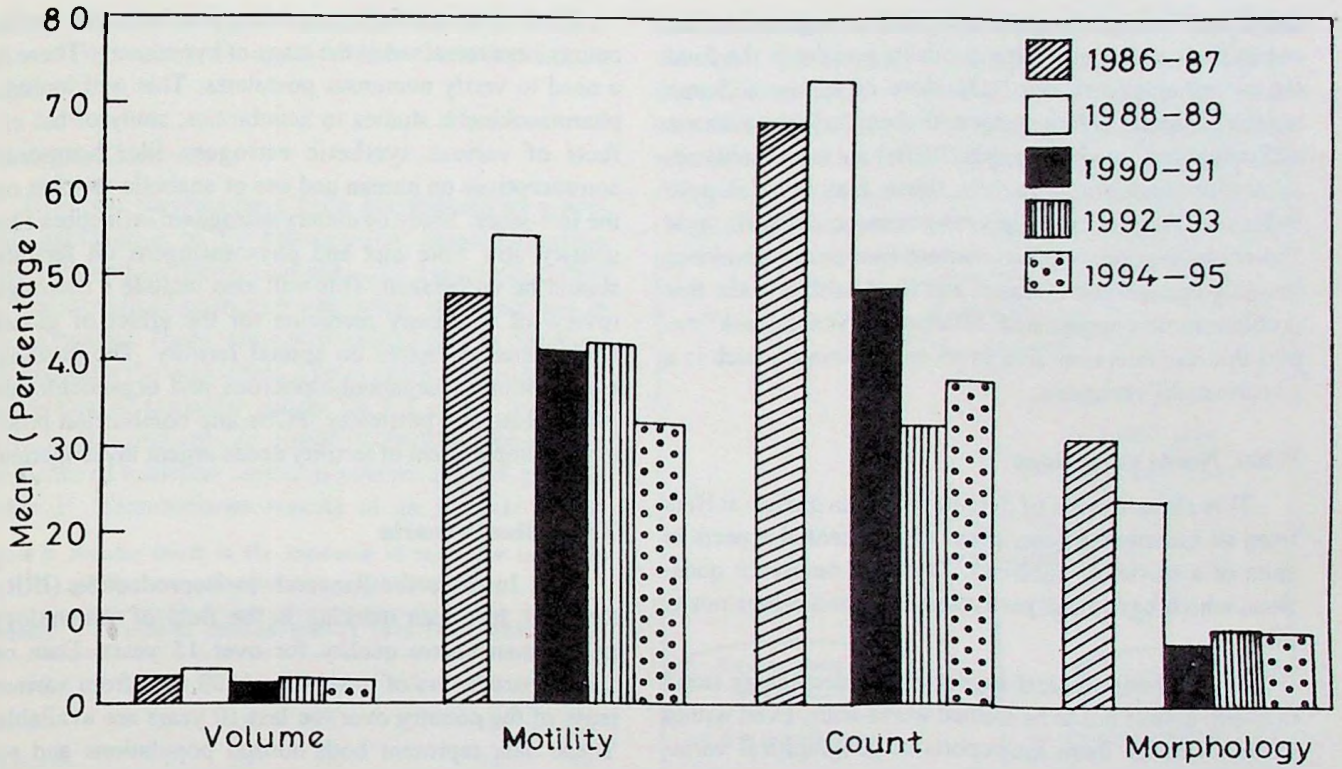



**Fig. 2. Changes in seminal parameters (mean) from 1986 to 1995**

reported to be the cause of pseudohermaphroditism in marine gastropod species[41-43]. One is very well aware of the chemical and oil spills in sea and how they affect the flora and fauna. For example a chemical spill of pollutants (compounds with estrogenic and endocrine disrupting activities) into lake Apopka, Florida, USA had resulted in significant decrease in the number of juvenile alligators. These alligators had abnormal testicular architecture and small phali and low testosterone concentrations[44]. Reports of morbidity and reduced male reproductive function (lowered testosterone, reduced gonadal size, hypothalamopituitary-gonal axis disturbances) have been reported in male fish living in the effluent waters of different industries[45]. The DES data on humans have been confirmed by the animal studies. These studies have shown an increased incidence of cryptorchidism, urethral abnormalities, testicular hypoplacia and infertility. When other synthetic estrogens were tested similar effects were seen . The animal studies have indicated that treatment of pregnant rats with chemicals with known estrogenic effects led to reduction of testicular size in the male offspring[32].

## Conclusion

There is a need for a critical comprehensive study of this phenomenon to establish it as a scientific fact. There are ethical and emotional issues contingent on the truth regarding the declining sperm counts as it challenges the basic survival of the human race. The risk of over reacting in this case has less serious consequences than ignoring any threat to male reproductive function. This is one issue where none of us can afford to do too little too late.

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This write-up has been contributed by Dr Kamala Gopalkrishnan, Deputy Director, Institute for Research in Reproduction, Mumbai.

# ABSTRACTS

## Some Research Projects Completed Recently

### Detection and assessment of molecular markers associated with multi-drug resistance in high grade non-Hodgkin's lymphoma

Lymph node biopsies and fine needle aspirates from 109 patients with high grade non-Hodgkins lymphoma (NHL) were analysed to study the expression of mdr gene and mdr gene product–P-glycoprotein in high grade NHL and correlation of the findings with the clinical manifestations and course of the disease. Normal lymph nodes dissected during head and neck surgery were used as control. Patients with severe disease at presentation, those with a second malignancy or coming after treatment at another hospital, and those over the age of 70 years were excluded from the study. The patients were clinically followed up for periods ranging from 7 to 34 months after chemotherapy or until the recurrence of the disease.

Of the 109 patients, 73 remained disease free during the follow up period and 36 patients showed residual or recurrent disease. Patients with residual or recurrent disease underwent a second lymph node biopsy analysis. None of the control lymph nodes and only 5 of the 73 (7%) patients who remained disease free during the follow up period showed the presence of P-glycoprotein. However, 26 of the 36 patients (72%) with recurrent/ residual disease expressed P-glycoprotein suggesting a correlation between the expression of P-glycoprotein and recurrence of disease.

*In situ* hybridization using a specific C-DNA probe to the mdr gene gave a positive reaction in 2 of the 5 disease free patients who showed expression of P-glycoprotein but in none of the 68 disease free patients with no expression of P-glycoprotein. Twenty five of the 36 patients with residual/recurrent disease showed the expression of mdr gene. Western blot analysis showed 30 of the 36 samples from these patients to be positive for P-glycoprotein, while Southern blot analysis for mdr gene showed positive reactions in all the 36 samples. None of the samples from 42 of the 73 disease free patients gave positive Western blot or Southern blot reaction. Repeat biopsies from all the 36 patients with residual/recurrent disease gave positive Western blot and Southern blot reactions.

Correlation analysis showed significant relationship between prognosis and the detection of P-glycoprotein by immunocytochemistry and Western blot and mdr gene expression by Southern blot. P-glycoprotein analysis therefore is a prognostic indicator for high grade NHL provided it is accompanied by Western blot and Southern blot analysis to rule out false negative.

M. Radhakrishna Pillai
Division of Laboratory Medicine and
Tumour Biology,
Regional Cancer Centre
Thiruvanantapuram

**Characterization and evaluation of hydatid antigens with diagnostic potential in early asymptomatic hydatidosis**

The study was carried out for identification and characterization of purified antigens from hydatid cyst and for identification of immunoreactive hydatid fluid antigenic fractions by Western blotting which could ultimately be used for the early diagnosis of hydatidosis and to study the kinetics of immune response in experimentally infected animal model.

Hydatid cyst fluid collected from hydatid cysts of naturally infected sheep was processed for the preparation of crude soluble hydatid fluid antigen by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE analysis revealed multiple protein bands of molecular weight ranging from 8 to 200 kDa. Of these, most demonstrable protein fractions occurred as a thick band in the range of 66-90 kDa.

Western blot analysis of hydatid cyst fluid with serum samples from patients of hydatidosis, neurocysticercosis, amoebiasis, intestinal helminthic infection and normal healthy individuals indicated the presence of multiple immunoreactive antigenic components ranging from 8 to 200 kDa. Of these, 8 and 45 kDa antigenic fractions were found to be specific for the diagnosis of human hydatidosis. Sensitivity and specificity of enzyme linked-immunosorbent assay (ELISA) for the detection of specific hydatid antibodies in optimally diluted (1:1280) serum samples of patients clinically suspected to have hydatidosis was found to be 81.8 and 100 per cent respectively.

Experimental infection could be established in mice by intraperitoneal inoculation of 2000 protoscolices of hydatid cysts obtained from naturally infected sheep. Kinetics of immune response to the crude soluble extract antigen revealed positive response by the second month post infection which persisted till the sixth month post infection (follow up for six months). Crude soluble extract antigen was found to be more suitable than the purified antigenic fraction for assessing immune response in early hydatidosis.

N. Malla
Department of Parasitology,
Postgraduate Institute of Medical
Education & Research, Chandigarh

## ICMR NEWS

Smt. Renuka Chowdhury, Hon'ble Minister of State for Health and Family Welfare, presented the ICMR Awards and Prizes for 1995 to outstanding biomedical scientists on August 22, 1997. She also launched the ICMR celebrations of the 50th year of Independence of India.

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Dr. G.V. Satyavati, a well known pharmacologist (with specialization in Medicinal Plants/Indigenous Drugs/Ancient Systems of Medicine) and biomedical information and communication scientist, who took over as the Director-General, ICMR, on May 7, 1994 retired on August 24, 1997 (on superannuation) after distinguished service of over three decades in the ICMR. Dr. Satyavati had the unique distinction of being the first woman to head not only the ICMR but any scientific/research agency in independent India.

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

**Meetings of the Project Review Committee (PRC)/Task Force (TF) held in New Delhi :**

| | |
|---|---|
| PRC on Biochemistry and Cellular & Molecular Biology | July 27, 1997 |
| Combined meeting of the Investigators and Steering Committee of the TF on monitoring of adverse drug reactions in India | August 8, 1997 |

**Meetings of the Scientific Advisory Committee (SAC)/ Ethical Committee :**

| | |
|---|---|
| SAC of the Centre for Advanced Research for Bone Marrow Transplantation for Thalassaemia, Vellore | July 31, 1997 (at Vellore) |
| Central Ethical Committee of ICMR on Human Research | August 10, 1997 (at New Delhi) |

**Participation of ICMR Scientists in Scientific Events:**

Dr. Bela Shah, Senior Dy. Director-General, ICMR, participated in the International Conference on Preventive Cardiology at Montreal (June 29-July 3, 1997).

Dr. V. Ramesh Bhat, Dy. Director (Senior Grade), National Institute of Nutrition (NIN), Hyderabad, participated in the meeting of the FAO/IAEA Consultant on Food Contaminants at Vienna (July 14-18, 1997).

Dr. P.K. Das, Director; Dr. K.N. Panicker, Dy. Director (Senior Grade); and Dr. S.P. Pani, Dy. Director, Vector Control Research Centre, Pondicherry, participated in the II International meeting on the Control of Lymphatic Filariasis at Townsville, Australia (July 18-20, 1997).

Dr. G.B. Nair, Dy. Director, National Institute of Cholera and Enteric Diseases, Calcutta, participated in the Executive Board meeting of the International Union of Microbiological Societies at Sydney (July 25-27, 1997).

Dr. A.R. Agarwal, Senior Research Officer, Institute for Research in Medical Statistics, New Delhi, participated in the III International Conference on Combinatorics, Information Theory and Statistics at Portland, Oregon (July 18-20, 1997).

Dr. Kamala Krishnaswamy, Director and Y. Venkataramana, Research Officer, NIN, Hyderabad, participated in the XVI International Congress of Nutrition at Montreal (July 27-August 1, 1997).

Dr. P.K. Meherji, Dy. Director and Dr. Jyotsna Gokral, Research Officer, Institute for Research in Reproduction, Mumbai, participated in the XV FIGO World Congress of Gynaecology and Obstetrics at Copenhagen (August 3-8, 1997).

Dr. S.K. Subbarao, Dy. Director (Senior Grade), Malaria Research Centre, Delhi, participated in the WHO Intercountry Workshop on Insecticide Resistance of Mosquito Vectors at Salatiga, Indonesia (August 5-8, 1997).

## ICMR AIDED SYMPOSIA/SEMINARS/WORKSHOPS/COURSES/CONFERENCES

| Symposium/Seminar/Workshop/ Course/Conference | Date & Place | Contact Address |
|---|---|---|
| International Symposium on Brain Ageing | August 9, 1997; (at New Delhi) | Dr. A.B. Dey, Addl. Professor, Department of Medicine, All India Institute of Medical Sciences, New Delhi-110029. |
| Symposium on Gastrointestinal Infection and Inflammation | August 29-30, 1997; (at Vellore) | Dr. B.S. Ramakrishna, Organising Secretary of the Symposium, Department of Gastroenterology, Christian Medical College, Vellore-632002. |
| III National Conference of the Alzheimer's and Related Disorders Society of India. | August 31, 1997; (at Hyderabad) | Mr. K.R. Gangadharan, Organising Secretary of the Conference, Alzheimer's and Related Disorders Society of India, 7-1-59/4 & 8, Ameerpet, Hyderabad-500016. |
| Symposium on Recent Trends in Tropical Disease Research | September 5-6, 1997; (at Sevagram) | Dr. M.V.R. Reddy, Organising Secretary of the Symposium, J.B. Tropical Disease Research Centre and Department of Biochemistry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha-442102. |
| National Symposium-cum-Workshop on Leptospirosis | September 11-13, 1997; (at Port Blair) | Dr. V.G. Rao, Organising Secretary of the Symposium, Regional Medical Research Centre, Port Blair-744101. |
| International Symposium on Recent Trends in Mental Health | September 19-20, 1997; (at Sevagram) | Dr. Prakash D. Behere, Organising Secretary of the Symposium, Department of Psychiatry, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha-442102. |

| Symposium/Seminar/Workshop/ Course/Conference | Date & Place | Contact Address |
|---|---|---|
| Mid Term Conference of the Indian Society for Clinical Pharmacology and Therapeutics | September 19-20, 1997; (at Mumbai) | Dr. S.R. Shahani, Organising Secretary of the Conference, Department of Pharmacology, L.T.M. Medical College, Mumbai-400022. |
| International Symposium on Free Radicals in Medicine | September 22-24, 1997; (at Udaipur) | Dr. P.P. Singh, Organising Secretary of the Symposium, Department of Biochemistry, R.N.T. Medical College, Udaipur-313001. |
| IV National Conference of the Society of Biomaterials and Artificial Organs on New Horizons in Biomaterials Research | October 3-4, 1997; (at Hyderabad) | Dr. P.R. Chatterjee, Convenor of the Conference, Indian Institute of Chemical Technology, Hyderabad-500007. |
| IX National Congress of Veterinary Parasitology | October 6-8, 1997; (at Ludhiana) | Dr. H.S. Bali, Organising Secretary of the Congress, Department of Veterinary Parasitology, College of Veterinary Science, Punjab Agricultural University, Ludhiana-141004. |
| Guha Research Conference-1997 | October 13-17, 1997; (at Palampur) | Dr. Amit Ghosh, Co-convenor-GRC'97, Institute of Microbial Technology, Chandigarh-160014. |
| Seminar on Current Status of Thalassaemia in India and National Workshop on Carrier Detection and Antenatal Diagnosis of Thalassaemia | November 13 and 14-15, 1997; (at Lucknow) | Dr. Sarita Agarwal, Organising Secretary, Department of Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow-226014. |
| International Conference on Advances in Reproductive Medicine | November 15-18, 1997; (at Calcutta) | Dr. Sudersan Ghosh Dastidar, Organising Secretary-ICARM'97, Infertility Clinic and I.V.F. Centre, 208, Rash Behari Avenue, Calcutta-700029. |
| Asia-Pacific Congress on Head and Neck Cancer | December 11-14, 1997; (at Mumbai) | Dr. Ashok R. Mehta, Chairman of the Congress, Department of Surgery, Tata Memorial Hospital, Mumbai-400012. |


## INDIAN COUNCIL OF MEDICAL RESEARCH

### Grant-in-aid for organising Seminars/Symposia/Workshops

The Council provides partial financial assistance for organising Seminars/Symposia/Workshops. Applications for grant of financial assistance (complete in all respects in the prescribed proforma), will be considered only if furnished **at least four months before the date of commencement of the Seminar/Symposium/Workshop,** *etc.*

## COUNCIL'S TRAINING PROGRAMMES FOR 1997-98

**Leprosy**

*At the Central JALMA Institute for Leprosy, Agra:*

- Training Course on National Leprosy Eradication Programme for Medical Officers (September 15-26, 1997).

**Reproductive Biology**

*At the Institute for Research in Reproduction, Mumbai:*

- Workshop on Genotoxicity (August 18-29, 1997).
- Workshop on Basic Molecular Biology for Clinicians (December 2-11, 1997).

**Endocrinology**

*At the National Institute of Nutrition, Hyderabad:*

- Annual Certificate Course on Endocrinological Techniques and their Applications (August 1-September 15, 1997).

**Nutrition**

*At the National Institute of Nutrition, Hyderabad:*

- Postgraduate Certificate Course in Nutrition (December 1, 1997-February 28, 1998).

**Occupational Health**

*At the National Institute of Occupational Health, Ahmedabad:*

- Orientation Course on Occupational Health for Industrial Medical Officers (September 30-October 11, 1997).
- Training Course on Environmental Hygiene Practice (November 10-14, 1997).

**Laboratory Animal Technology**

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

- Training Course for Laboratory Animal Supervisors (September 1-November 29, 1997).

**Haematology**

*At the Institute of Immunohaematology, Mumbai:*

- Training Course in Transfusion Medicine for Blood Bank Medical Officers (August 27-October 24, 1997).
- Training Course in Blood Group Serology and Blood Bank Methodology for Blood Bank Technicians (August 27-September 26, 1997).
- Training Course in Advanced Haematology and Immunohaematology (October 6-24, 1997).


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