

PERCEIVED REPRODUCTIVE MORBIDITY AND HEALTH CARE SEEKING BEHAVIOUR AMONG WOMEN IN AN URBAN SLUM

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ABSTRACT

This paper estimates the prevalence of various Reproductive Tract Infections in females in a slum of Delhi. All ever married women in the reproductive age group were interviewed by a pre-designed and pre-tested interview schedule. As many as 62.3 per cent reported one or more problems in the past six months. The risk factors like genital hygiene and abortions were significantly associated with reproductive morbidity. Out of the women who experienced a reproductive health problem, only 27.8 per cent consulted a health facility for management. The authors recommend effective services for RTIs/STIs coupled with an awareness generation for the better utilisation of these services in the urban slums.

Key-words: RTI/STI Risk factors, Health care seeking.

Sexually Transmitted Diseases (STDs) pose a public health problem of major significance in most parts of the world and South-East Asia was no exception. The STDs account for 1.9 per cent of the burden of disease in disability adjusted life years in females globally, the magnitude being maximum in South-East Asia and Africa¹. India has a high incidence of STDs with an estimated annual incidence rate of 5 per cent or 40 million new cases a year².

The focus in recent years has shifted from Sexually Transmitted Diseases to Reproductive Tract Infections / Sexually Transmitted Infections (RTIs/STIs). The latter took cognisance of the asymptomatic infections which were hitherto neglected. With the spread of the HIV/AIDS epidemic there has been a resurgence of concern about STI/RTI. Though RTI/STI were often researched in the setting of STD and gynaecology clinics, their profile in the general population remained elusive³⁻⁵. This was more so because only a small proportion of people experiencing symptoms of RTI/STI consider them as problems and sought

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antenatal and family planning clinic attendees has been taken as a proximate indicator of the magnitude of the problem in the population⁷⁻¹⁰. The community-based studies conducted have emphasised on elicitation of symptom complexes of reproductive morbidity for correct diagnosis¹¹⁻¹³.

The STD Control Programme has been in operation in India since 1946. Soon after the first case of AIDS was reported in India in 1986, efforts were made to combat the disease with the launching of the National AIDS Control Programme in 1987. The STD control was one of the main strategies in the prevention and control of HIV/AIDS and therefore, steps have also been undertaken to strengthen it^{1,14}. Apart from strengthening of STD clinics, efforts were also made to integrate STD case management in the primary health care delivery system, although this was not without inherent constraints in the diagnosis of STDs. Till the time, at least peripheral laboratory tests could routinely be incorporated at peripheral health facilities, the diagnosis and treatment of RTIs / STIs has to rely up on the symptomatology. Although, some studies have shown variability in correlation of self-reported versus clinical examination and laboratory tests, but resource poor countries have endorsed the syndromic approach for treatment of RTI/STI^{15,16}.

Efforts to operationalise the syndromic approach for the treatment of RTI/STI need an assessment of the magnitude of the prevalence of the symptom complexes in the community. In the last decade, considerable work has been done to determine the load of gynaecological morbidity^{12,13,15,17-20}. Such studies were required not only for logistic management but also for determining the quantum of workload to be assigned to health care functionaries at different levels, including referral facilities. Surveillance of STDs formed an important component of the National AIDS Control Programme in India and was based on the collection of Syndrome-based information through peripheral health institutions.⁶ In India although, there has been a well defined system of primary health care delivery in the rural areas but the urban set-up was characterised by a multiplicity of agencies with wide variation in availability of services. The scenario in the urban slums, which were not only characterised by a migrant and high risk population but also lacked an organised system of health care delivery, was the worst part in most of the cities. The present study was undertaken with the following objectives in view:

- i) To estimate the prevalence of Reproductive Tract Infections by symptom complexes in a slum population of Delhi;
- ii) To study the risk factors associated with RTI; and
- iii) To study the health care seeking behaviour of urban population in relation to RTI.

MATERIALS AND METHOD

Delhi, the capital of India has a population of approximately 14 million. It has a heterogeneous mix of population hailing from different parts of the country. Migration from rural areas of nearby States has grown at a fast pace in recent years. The main reason for this phenomenon was the manifold increase in employment opportunities that the migrants were getting in the cities. However, due to densely crowded physical habitation, the urban slums in which they settle lack the basic infrastructure, sanitation and hygiene. As per an estimation 40 per cent of the population of Delhi has been residing in slums²¹.

The present study was conducted in a slum - Balmiki Basti, located in the vicinity of Maulana Azad Medical College. The slum has been in existence for the past fifteen years. The approximate population of the slum is 1900. The source of maternal and child health services was a nearby health centre, being run by the city municipality. A practitioner of traditional system of medicine was practising in the area. The area has been recently adopted by the department of Community Medicine of Maulana Azad Medical College, New Delhi, as a field practice area for the delivery of primary health care. The health facilities were provided by the peripheral health post, Basti Vikas Kendra (Slum Development Centre) and a non-government organisation (Sur Nirman Educational and Cultural Society).

A demographic survey was conducted in the area when it was adopted by the Department of Community Medicine. It revealed that the area consisted mainly of people who had migrated from the States of Uttar Pradesh and Bihar. Eighty per cent of the families had income less than Rs. 2000 (\$ 42) per month. The men were generally engaged in unskilled or semi-skilled work while the women were mostly housewives. The water supply was through a piped system and sanitary latrines were being maintained by Sulabh International, a non-government organisation.

The study was carried out from July to December 2000. The units of study were all ever-married women in the reproductive age group of 15 to 45 years. A total of 231 married women (15-45 years) identified in the demographic survey were interviewed, using a semi-structured interview schedule.

Data thus collected were coded and analysed, using Epi info 6.04 package. Chi-square and Fisher's Exact test were also applied wherever applicable.

FINDINGS

Reproductive Profile

Out of a total of 231 married women, 115 (49.8 %) were between 20 and 30 years of age and married for 10 years or less. Of all the married women, duration of married life ranged from 1 to 35 years. The total number of pregnancies the women experienced ranged from 0 to 12. Of the respondents, 9.5 per cent were nulliparous while 69.3 per cent had 1 to 4 children. At the time of the data collection 29 (12.6 %) women were found to be pregnant (Table 1).

TABLE 1
CHARACTERISTICS OF THE STUDY PARTICIPANTS (N=231)

Characteristic	Mean (Standard Deviation)
Age (years)	29.3 (7.5)
Duration of marriage (years)	11.8 (7.7)
Pregnancies per woman	3.7 (2.5)
Live births per woman	3.0 (2.2)
Abortions per woman	1.6 (1.0)

Self-Reported Reproductive Morbidity

As many as 144 (62.3 %) reported one or more problems in the past six months. The mean number of problems per woman was 1.02 (SD 1.04). The problems reported most frequently were low backache (51.1%), pain during menstruation (41.1 %) and vaginal discharge (31.6 %) Table 2.

Symptom Complexes of Reproductive Tract Infections/Sexually Transmitted Infections

The symptoms reported by the women were grouped into symptom complexes and a presumptive diagnosis was made. If vaginal discharge with or without smell and/or genital itching and/or redness of genitalia were reported, then a presumptive diagnosis of vaginitis was made. Low backache, lower abdominal pain with fever or vaginal discharge and deep pain during sex were taken to indicate pelvic inflammatory disease. Burning micturition and painful swelling on the groin was taken to be indicative of urinary tract infection and inguinal bubo respectively.

TABLE 2
SELF-REPORTED REPRODUCTIVE HEALTH PROBLEMS (N=231)

Problem	Number	Percentage
Burning micturition	29	12.6
Vaginal discharge (Wetting/staining of underclothes/ with or without unpleasant smell)	73	31.6
Genital itching	30	13.0
Redness of genitalia	9	3.9
Painful swelling on the groin	5	2.2
Ulcers on the genitalia	4	1.7
Pain during menstruation	95	41.1
Low backache	118	51.1
Lower abdominal pain with fever or vaginal discharge	21	9.1
Deep pain during sex	13	5.6
Inability to conceive	14	6.1
Any problem	144	62.3

TABLE 3
SYMPTOM COMPLEXES IN THE STUDY SUBJECTS (N=231)

Problem	Number	Percentage
Vaginitis	76	32.9
Pelvic Inflammatory Disease	122	52.8
Urinary Tract Infection	29	12.6
Inguinal Bubo	5	2.2
Genital Ulcer	4	1.7
Infertility	14	6.1

The presumptive diagnosis made was then examined in relation to the hypothesised risk factors. The three diagnosis of urinary tract infection, vaginitis and pelvic inflammatory disease only had frequencies large enough to be examined in relation to risk factors (Table 3).

Self-Reported Morbidity Versus Variables of Reproductive Profile

Among the women married for more than 10 years, pelvic inflammatory disease was observed to be higher than those married for less than 10 years. Women with more than seven pregnancies were found to have more prevalence of PID. The number of pregnancies and live births was not found to be associated with the presence of either of the three symptom complexes. The history of abortions was significantly related to the symptom complexes of vaginitis ($p=0.01$) and Pelvic Inflammatory Disease ($p<0.05$) (Table 4).

TABLE 4
SELF-REPORTED REPRODUCTIVE MORBIDITY VS. REPRODUCTIVE PROFILE

Reproductive Profile	No	Urinary Tract Infection		Vaginitis		Pelvic Inflammatory Disease	
		No.	Per cent	No.	Per cent	No.	Per cent
Marriage Duration							
1 – 10 yrs	115	17	14.8	39	33.9	56	48.7
>10yrs	116	12	10.3	37	31.9	66	56.9
Number of Pregnancies							
0 - 3	129	17	13.2	36	27.9	69	53.5
4 - 7	79	11	13.9	32	40.5	39	49.4
8 - 11	23	1	4.3	8	34.8	14	60.9
Live Births							
0 - 3	148	19	12.8	43	29.1	80	54.1
4 - 7	72	10	13.9	30	41.7	37	51.4
8 - 11	11	0	0.0	3	27.3	5	45.5

Genital/Menstrual Hygiene

All the three symptom complexes were found to be more in women not washing genitals daily. A statistically significant association was found between poor genital hygiene and vaginitis ($p<0.05$). Women were asked about the material used during menstruation. Any cloth was taken to be unclean for the purpose of examining the association of menstrual hygiene with RTIs while new cloth, cotton-wool and branded napkin were taken to be clean. Higher prevalence of morbidities was found in women using unclean soaking material during menstruation but it was not of any statistical significance. No association was found between frequency of changing soaking material and prevalence of symptom complexes.

Contraceptive Usage Versus Symptom Complexes of Reproductive Morbidity

Of the study respondents 108 (46.8 per cent) had used one or more contraceptives in the past. Women who had adopted a permanent method of contraception was 51(21.1 per cent).Tubectomy was much more popular among women (20.3%). Condoms, intrauterine device and oral pills had been used by 28 (12.1 per cent), 28 (12.1 per cent) and 16 (6.9 per cent) women respectively. The women who had used a temporary method before opting for sterilization was 8(3.5 per cent) while 6 (2.6 per cent) women had used more than one temporary methods. The duration of use was one year or less for 22 (9.5 per cent), one to three years for 26 (11.3 per cent) and more than three years for 60 (25.9 per cent) respondents. The prevalence of symptom complexes were lower in ever

users of condoms although the difference was not statistically significant. Ever users of intrauterine device were found to have a higher prevalence of all symptom complexes than non-users but it reached statistical significance only in case of Pelvic Inflammatory Disease.

Health Care Seeking

Out of the 144 women who experienced one or more reproductive health problems only 40 (27.8%) consulted a health facility for treatment. The health care seeking was maximum for inguinal bubo (60%) followed by genital ulcer (50%) and urinary tract infection (48.3%). Of the women diagnosed to have vaginitis, 34.2 per cent and pelvic inflammatory disease 24.6 per cent sought treatment, Table 5.

TABLE 5
HEALTH CARE SEEKING OF WOMEN ACCORDING TO THEIR
REPRODUCTIVE HEALTH PROBLEMS

Problem	N	Number seeking care	Percent
Vaginitis	76	26	34.2
Pelvic Inflammatory Disease	122	30	24.6
Urinary Tract Infection	29	14	48.3
Inguinal Bubo	5	3	60.0
Genital Ulcer	4	2	50.0
Infertility	14	2	14.3
Any Problem	144	40	27.8

DISCUSSION

The National Family Health Survey-2 (NFHS-2)²² reports the proportion of women having any reproductive health problem to be 36.5 per cent in Delhi which is much lower than the present finding i.e., 62.3 per cent. Different community based studies from urban and rural areas in the Indian context found the prevalence of self-reported morbidity varying from 55 to 100 per cent^{12,13,15,17-20}. A study in an urban population of Istanbul reports a proportion of 65 per cent.²³ The differences could be attributed to the socio-demographic characteristics like education and standard of living because the study area was a slum population. These variables have been found to have an association with the prevalence of reproductive health problems.²² Findings of the present study indicate a higher prevalence of most problems, especially backache and burning micturition, as compared to the other studies from urban India.¹³ Complaints of vaginal discharge, burning micturition and dysmenorrhoea were found to be significantly higher than those in rural Indian communities¹⁷.

It was observed that the women in the extremes of the reproductive age group have lower prevalence of vaginitis and PID, a finding similar to NFHS-2

wherein it was found that the symptoms suggestive of RTI were more in the age group of 25 to 39 years²². Duration of marriage, gravidity, parity and number of abortions were not found to be related to reporting of symptoms of RTI/STI. Gravidity was found to be a significant associate of gynaecological problems¹². History of abortions was found to be a significant predictor of occurrence of vaginitis and PID. Though majority of the women reported having taken services of a government facility whether for inducing abortions or for evacuation of spontaneous abortion the post-abortion care could be lacking predisposing the women to infection. Women who did not maintain perineal hygiene were more likely to report lower reproductive tract infections¹².

Since past contraceptive use has been hypothesised to have an association with prevalence of RTI, the association of ever use of contraceptive was examined for the prevalence of RTI. Presence of RTI symptom was found to have a significant association with contraceptive use¹⁷. While no significant difference in the proportion of women suffering from Urinary Tract Infection and Vaginitis among users and non-users was observed. Prevalence of Pelvic Inflammatory Disease was found to be significantly higher in ever users of contraceptives. Use of intrauterine device and sterilisation was found to be significant predictors of symptoms of RTI/STI in the present study while condoms offered protection¹⁵. Oral contraceptive users were found to have higher prevalence of all the three infections which disprove the popular belief that oral contraceptives provide protection against PID. Intrauterine device use was also associated with a higher prevalence of all three infections while it was significant only in case of PID. This could be due to insertion of intrauterine device under unhygienic conditions or lack of proper screening before insertion of IUD. Higher prevalence of infection was observed among women who had undergone sterilisation^{12,15}.

As per the study findings, only 27.8 per cent of those experiencing a reproductive health problem took treatment. This was much lower as compared to NFHS-2 (44.7%).²² The proportion of seeking health care varied from 25 per cent for PID to 60 per cent for inguinal bubo. This was in spite of the services of a tertiary care hospital attached to the medical college being available in the vicinity of the slum. This was again a pointer to the fact that reproductive health problems were not perceived as abnormal and requiring medical attention. Only 2 (14.3%) of the women suffering from infertility sought care which was found in contrast to other studies.¹⁵ This could be attributed to the fact that these two women were suffering from primary infertility in contrast to the other 12 who had already given birth to at least two children and proven their fertility..

CONCLUSION

The high prevalence of self-perceived reproductive morbidity among women in the urban poor setting highlights the need for implementation of a well-conceived intervention programme. Preventive strategies with regard to

reproductive morbidity and promotion of health care seeking behaviour among the women will help in reducing the magnitude of the problem in the study area.

REFERENCES

1. WORLD HEALTH ORGANIZATION (2000): The World Health Report, Health Systems: Improving Performance.
2. NATIONAL AIDS CONTROL ORGANISATION (2000): Ministry of Health and Family Welfare, Government of India, Specialist's Training and Reference Module.
3. JAYA SINGH P., RAMNAIAH T.B. AND FERNANDEZ S.D. (1985): Patterns of STDs in Madurai, India; *Genitourinary Medicine*, 61, p. 99:103.

4. MITTAL A. AND KAPOOR S. (1998): Screening for Genital Infection in Symptomatic Women; *Indian Journal of Medical Research*, 93, p. 119-123.
5. BHALLA P. AND KAUSHIKA A. (1994): Epidemiological and Microbiological Correlates of Bacterial Vaginosis; *Indian Journal of Dermatology, Venereology and Leprology*; 60, p. 8-14.
6. NATIONAL AIDS CONTROL ORGANISATION (1999-2000): Ministry of Health and Family Welfare, Government of India, Combating HIV/AIDS in India.
7. WEISSENBERGER R., ROBERTSON A., HOLLAND S. ET AL (1977): The Incidence of Gonorrhoea in Urban Rhodesian Black Women; *South African Medical Journal*; 52, p. 119.
8. NASAH B.T., NGUEMATCHA R., EYONG M. AND GODWIN S. (1980): Gonorrhoea, Trichomonas and Candida among Gravid and Nongravid Women in Cameroon; *International Journal of Gynaecology and Obstetrics*, 18, p. 48-52.
9. RATNAM A.V., DIN S.N. AND HIRA S.K. ET AL (1982): Syphilis in Pregnant Women in Zambia, *British Journal of Venereal Diseases*; 58, p. 355.
10. O'FARRELL N.O., HOUSEN A.A., KHARSANY B.M. AND VAN DEN ENDE J. (1989): Sexually Transmitted Pathogens in Pregnant Women in a Rural South African Community; *Genitourinary Medicine*, 65, p. 276-280.
11. HUDA Z., HIND K., NABIL Y., OLFIA K. AND MAHINAZ E.H. (1995): Comparing Women's Reports on Medical Diagnosis of Reproductive Morbidity Conditions in Rural Egypt; *Studies in Family Planning*, 26(1), p. 14-21.
12. BHATIA J.C. AND CLELAND J. (1995): Self-Reported Symptoms of Gynaecological Morbidity and their Treatment in South India; *Studies in Family Planning*, 26 (4), p. 203-216.
13. LATHA K., KANANI S.J., MAITRA N., BHATT R.V., SENAPATI S.K., BHATTACHARYA S., SRIDHAR S., GIRI G.B., SHAH P.P., SHAH S.P., DESAI L.A., PARIKH I., TASKAR V., DHARAP N. AND MULGAONKAR V. (1997): Prevalence of Clinically Detectable Gynaecological Morbidity in India: Result of Four Community Based Studies; *The Journal of Family Welfare*; 43(4), p. 8-16.
14. NATIONAL AIDS CONTROL ORGANISATION, Ministry of Health and Family Welfare, Government of India, Family Health Awareness Campaign, Operational Guide.

15. GARG S. (2000): An Epidemiological and Sociological Study of Symptomatic and Asymptomatic Reproductive Tract Infections and Sexually Transmitted Infections among Women in an Urban Slum; Task Force Project Under Special Programme of Research, Development and Training.
16. HAWKES S.L., MORRISSON S., FOSTER, K. GAUSIA, CHAKARABORTY J. AND WEELING R. (1999): Reproductive Tract Infections in Women in Low Income Low Prevalence Situations: Assessment of Syndromic Management in Matlab, Bangladesh; *The Lancet*, 354, p.1776-81.
17. BANG R.A., BANG A.T., BAITULE M., CHOUDHARY Y., SARMUKADDAM S. AND TALE O. (1989): High Prevalence of Gynaecological Diseases in Rural Indian Women; *Lancet*; Jan. 14, p. 85-88.
18. OOMMAN, N. M. (1996): Poverty and Pathology: Comparing Rural Rajasthani Women's Ethnomedical Models with Biomedical Model of Reproductive Behaviour; *Ph.D. Thesis, Johns Hopkins University, Bethesda Maryland (U.S.A.)*.
19. PRASAD J.H., GEOGE V., LALITHA M.K., JAYAPUL M.N.R., ABRAHAM S., SHETTYAND N., JOSEPH A. (1999): Prevalence of Reproductive Tract Infections among Adolescents in a Rural Community in Tamil Nadu; *Unpublished paper*.
20. SHENOY K.T., SHENOY S. AND GOPALAKRISHNAN K. (1997): Reproductive Health and Gynaecological Morbidity in Kerala; *UNDP Draft Report*.
21. Synthesis Report of the UBSP Benchmark Survey (1997): National Institute of Urban Affairs, Vol. 1 and 2.
22. INTERNATIONAL INSTITUTE FOR POPULATION SCIENCES (1998-99): National Family Health Survey-2.

A STUDY OF CLEANING/DISINFECTING PROCEDURES IN A PREMIER TERTIARY CARE HOSPITAL, DELHI

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ABSTRACT

This study was undertaken for a short period of 3 weeks in a premier tertiary care hospital to analyse the housekeeping products and equipment used for environmental sanitation. Simultaneously, the techniques adopted to disinfect the various patient care areas were also studied. The type of disinfectant, detergent used and techniques of their dilution-ratio were also studied.

Key-words: Swabs, Disinfectants, Washing

Environmental cleanliness (housekeeping) is a part of the infection control programme of the hospital. However, there are no laid down norms for environmental cleanliness in the hospitals. Even the presence of microbes in the air has been demonstrated by several studies. Therefore, it was felt necessary to develop a standard for cleaning practices in a tertiary care super-speciality hospital.

Environmental survey in a premier tertiary care hospital was carried out by two methods. First, a volumetric air sampling which was done by exposing Mannitol Salt Agar (MSA) for one hour in the ward area to study the colony counts of Staphylococcus. The second method was to take swabs from surface of critical care equipment to study the bacterial growth. The Hospital Infection Control Committee (HICC) in the hospital has defined the bacterial growth of more than 15 colonies as significant⁹.

The Volumetric air sampling in the operation theatres and post operative surgical wards has shown more than 15 colonies of Staphylococci growth in MSA plates in 1999-2000. The Surface Swab Test of critical care equipment has shown a significant growth of Aerobic Spore Bearers (ASB), Staphylococcus Aureus (Staph) and Pseudomonas (Pseudo) (Table 1). The growth had increased in the year 2000 as compared to 1999.

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TABLE 1
SURFACE SWAB TEST OF CRITICAL CARE EQUIPMENT IN A
TERTIARY HOSPITAL

Critical Care Equipment in areas	Bacterial Growth	Colony Count in 1999	Colony Count in 2000
Main O.T.	ASB STAPH	55 29	65 50
Surgical Ward	ASB STAPH	40 16	59 36
Adult ICU	ASB STAPH PSEUDO	27 14 23	30 40 42
Paediatric ICU	ASB STAPH PSEUDO	32 - -	10 58 28
Medical Ward	ASB STAPH PSEUDO	50 25 38	70 64 43

In view of the above, a study of cleaning practices in the hospital was taken up with the following objectives:

- (i) To review the cleaning practices adopted in the hospital;
- (ii) To discuss the lacunae therein vis-a-vis standards laid by the Microbiologist world over; and
- (iii) To develop standard for hospital sanitation and housekeeping so as to reduce hospital infection.

Observation of Cleaning Practices in a Tertiary Level Hospital

A direct observational study was undertaken for three weeks in a premier tertiary care hospital to analyse the housekeeping products and equipment used for environmental sanitation. Simultaneously, the cleaning techniques adopted to disinfect the various patient care areas were also studied. The types of disinfectant, detergent used and techniques of their dilution ratio adopted for disinfection were examined. The observations thus made were also confirmed by the senior staff nurses, sanitary officers and members of hospital infection control committee.

Patient Care Areas: Most of the patient care areas were cleaned with brooms in the wards thrice a day by shifting the patients if possible or by asking them to cover their faces.

Wet mopping was also carried out thrice a day with detergent in 1:10 dilution at the onset of each shift of the sweepers. Spillage and isolation rooms with immunocompromised or HIV patients were cleaned with the wet mop. If more spillage was noticed then a disinfectant was poured on it and then wiped with a wet mop. The mop was then wringed dry after rinsing in water and stored

in the janitor closet. Generally, a mop was used for about 12-15 days and discarded. The mops were never laundered.

Bathrooms and Lavatories: Disinfection is carried out thrice a day with a phenolic disinfectant (diluting 350 ml in 2 liters of water) after cleaning the bathroom with wet mop impregnated in detergent and flushing the toilet with water. The sinks were cleaned once a day. Occasionally, bleaching powder (hypochlorite solution) has been used to clean the bathrooms and toilets.

Corridors and Stairwells: Corridors and stairwells were cleaned once a day directly with a wet mop. If required (as felt by the sanitary supervisor) they were cleaned for the second time with wet mop impregnated with detergent.

The wards, walls, bathrooms, lavatories and corridors were washed once a week by mixing soda and soft soap in 1:3 ratio. The solution was splashed in half of the ward first and then scrubbed from one end to the other with hard brush and cleaned with water. The same procedure was adopted in the rest of the half ward. Wet vacuuming was carried out once a week in all the patient care areas, corridors and stairwells. The walls were washed by pouring the mixture of soda and soft soap and then rinsed with water.

OT's and ICU's: Dry mopping and wet mopping have been done many times in a day mostly after every use. The walls were cleaned once every day and disinfected.

Carpet Care: There were no carpets in the hospital. Dry vacuuming was done in those office areas of the hospital where carpets have been provided.

Lacunae Detected

- The existing Hospital Infection Control Committee (HICC) laid more stress on cross infection in the wards than on environmental sanitation to reduce hospital born infection.
- The HICC policy manual has not provided any details about the policy on environmental sanitation nor it has given any description about the products, equipment and techniques to be used by the housekeeping department.
- The hospital infection control nurse took samples in air as a part of environmental survey for hospital infection. No technique has been used to survey the bacterial load on floors of ward areas, counters or table tops, etc.
- Broom has been used in most of the patient care areas which disperses dust and could be a problem to patients with respiratory disorders.
- The mop was neither washed in laundry nor with hot water rinsed with a chlorine-releasing agent before storing it dry in the janitor for the night.
- No chemical disinfectant has been used after cleaning the floors with detergent.
- Wet vacuuming was also not done daily. It was carried out once a week.

- No autoscrubbing, stripping or spray buffing was done to maintain the hard surfaces floors.

DISCUSSION

Cleaning Frequencies

Ideally, cleaning all areas should be done as soon as dirt becomes obvious, but flexible cleaning frequencies are impractical. Therefore, hospital policy for cleaning should be laid down very clearly in the hospital manuals. The major difference was in the frequency of cleaning of high-risk areas, like OT's and ICU's. Such areas should be cleaned more often.

The washing of walls and ceilings should be infrequent, not more often than once a month or two. However, where walls were splashed with blood or other organic material, the soiled patches should be washed as soon as possible. There was no evidence available that the use of chemical disinfectant justified the cleaning of walls as walls have very few microbes.

Cleaning Agents

A cleaning agent should assist the removal of dirt from a surface without giving harm to the user or damage to the surface. The following cleaning agents are suggested for use in the hospital^{1,2,10}.

- i. An anionic liquid detergent is found to be most suitable for floors, ceilings, walls, work surfaces and baths;
- ii. Scouring powder for cleaning sinks, baths, showers and lavatories;
- iii. The presence of disinfectant does not increase the cleaning power of the product; and
- iv. Chemical disinfectants are useful only on clean surfaces, therefore the floor should be first cleaned with detergent and then disinfected.

Selection of Disinfectants

Every hospital should have a Hospital Infection Control Committee, which should prepare the disinfectant policy and decide the types of disinfectants to be used^{1,2,6,10,16}.

Chemical Disinfectants

They are recommend for disinfection of baths after cleaning, disinfection of kitchen work surface after cleaning, disinfection of floors and work surfaces in OTs, special care units, high-risk areas during or after cleaning on some occasions and disinfection of floors after spillages^{13,14,16}.

Recommended Cleaning Techniques^{8,13}

Floors

In a busy ward, recontamination from airborne settlement or transfer from shoes and trolley wheels is very rapid. Levels of bacterial contamination on floors were usually restored to their original level within two hours of cleaning whether disinfectants were used or not. Infection rates were not influenced by the use of a disinfectant. Detergent alone can suffice. Disinfectants should only be used on a clean surface. However, mops should be disinfected after use in the rooms of infected patients and also before use in rooms occupied by immunosuppressed patients. Laundering in a machine with a heat disinfection cycle is a preferred method, but rinsing followed by a soak in (1%) bleach or chlorine releasing agent (1000 ppm av. Chlorine) for 30 minutes, re-rinsing and allowing to dry was an acceptable alternative.

A neglected dry mop will redistribute microbes, which have been picked up. A neglected wet mop will grow pseudomonas on it, which will get distributed again the next time while cleaning. A study by Isobel M. Maurer revealed that when both cotton and plastic mops were cleaned with water, the mops were heavily loaded with microbes when allowed to dry overnight¹⁵. The cleaning of mops after use in a disinfectant showed variable results whereas on boiling at 85°C after use and being left overnight, there was no growth. The author has further emphasised that there was no point in using a chemical disinfectant for routine floor cleaning, if floors were washed once a day.

Carpet Care

Usually bacteria have been found to be present in large numbers on the carpets and survive longer than on hard floors. However, there was no evidence that carpets were associated with infection. Therefore, the carpets in surgical and clinical areas should have waterproof backing and joints sealed. Pile fibers should be water repellent, non-absorbant, short and upright for easy cleaning. Carpets should be first tested with the commonly used disinfectants to see their damaging capacity. Chlorine releasing agents could be used to clean blood spillage, but it damages most carpets. Therefore, peroxygen powder could be used as a substitute. Spillage could also be removed by watering with a detergent solution. However, large spillage becomes, difficult to maintain because of smell of stain and hence carpets are not preferred in clinical areas. In offices and administrative areas carpets could be maintained with a daily vacuuming and shampooing once a week.

Spillage

Liquid disinfectants like phenolics or chlorine releasing agents could be used for spillage >30 ml. Chlorine releasing powder, granules or fluids containing

10,000 ppm av. chlorine, should be used for blood or body fluid spillage if contaminated with HBV or HIV.

The key to maintain a shining floor was the creation of a perfectly smooth surface that increases the reflection of light from floor.

Maintenance of hard surface floor includes dry mopping, wet mopping, auto scrubbing, stripping, spray buffing or brushing. A good floor care is a combination of all the above factors. The stripping operation is meant for complete finish removal and could be damaging in the long run because of use of alkaline solution. If the floor has not been discoloured or damaged, 1 or 2 layers of finish should be removed. Spray buffing is done on hard floors to increase the shine by smoothing the surface.

Walls and Ceilings

It should be carried out sufficiently often to prevent the accumulation of visible dirt, but intervals between cleaning should not exceed 12-24 months in patient care areas or 6 months in operating theaters.

Bathroom and Lavatory Cleaning

A neglected bathroom not only becomes unhygienic but also provides an advertisement to the visitors and the staff members indicating that the housekeeping department was not doing a good job.

A study by St. Francis Hospital showed that spray bottle method was the best for cleaning bathrooms. Here, sufficient amount of disinfectant solution could be sprayed on to the bathroom fixtures and surfaces and be allowed to remain for a period of maximum disinfection. Another study by Isobel M. Maurer observed that the cleaning and flushing of toilet was enough to keep the microbes' level to the minimum. Storage of lavatory brushes in solutions of chemical disinfectants was not recommended as the brushes inactivate them. Brushes rinsed with water and shaken into the pan and stored dry was enough. Baths could be a source of cross infection. Therefore, Isobel M. Maurer has suggested the use of scouring powder or a liquid detergent (preferably anionic with hypochlorite solution) for thorough cleaning after use. Daily use of bowl cleaners were not recommended as they were acid and need to be handled carefully by an expert. It was more cost effective and safer to use a disinfectant detergent to clean toilet on a daily basis, saving the bowl cleaners for use on mineral deposits only.

Ancillary areas

This includes offices, solaria and waiting room. In a general sense, the same rule of cleaning is applied here also but in a much rigorous way with a lesser frequency. The objective is to provide a clean and safe environment.

Corridors and Stairwells

Two important things to be obeyed while working on stairwells and corridors were- first to use wet floor signs if the floor is wet and second, to mop only one-half of a corridor or stairwell at a time to avoid accidents.

Kitchen Work Surface

All disinfectants get inactivated to some extent by food, particularly proteins. A two-step process of cleaning with a detergent and then with disinfectant obtains the best results.

LIMITATIONS

The study was undertaken for a short period of six weeks. Three weeks were spent to review the available literature while rest of three weeks were devoted to observation along with interview of the concerned staff to confirm the techniques adopted.

IMPLICATIONS

The findings of the study can be utilised by various hospitals to reduce their hospital acquired infection rates as well as to improve the environmental cleanliness.

REFERENCES

1. AYLIFFE G.A.J., D. AND HOFFMAN P.N. (1984): Chemical Disinfection in Hospital, Public Health Laboratory Service, London.
2. BENNETT V., JOHN BRACHMAN. AND PHILLIP S. (1998): Hospital Infections; 4th Edition, *Lippincott – Raven Publisher*, Philadelphia.
3. BLOOMFIELD S.F. AND MILLER E.A. (1989): A Comparison of Hypochlorite and Phenolic Disinfectants for Disinfection of Clean and Soiled Surfaces and Blood Spillages, *Journal of Hospital Infection*, (13), p. 231.
4. COATES D. (1988): Comparison of Sod. Hypochlorite and Sod. Dichoroisocyanurate Disinfectants: Neutralisation of Serum, *Journal of Hospital Infection*, (11), p. 60.
5. COUNCIL OF EUROPE (1987): Test Methods for the Antibacterial Activity of Disinfectants, Stanburg.
6. DAVID D. GURALNIK (1980): Websters' New World Dictionary USA; Editorial, *The World Publishing Company Inc.*, p.384.
7. GREEN V.W., VESLAY D., BOND R.G. AND MICHAELSEN G.S. (1962): Microbiological Studies of Hospital Air: Qualitative Studies; *Appl. Microbiol.* (10), p. 567.
8. HANDBOOK OF CONTACT FLOOR CONTRACT FLOOR COVERING, 1981-82 Edition, (1983): *Hearst Business Communications Inc.*, FCW Decision, New York.
9. HOSPITAL INFECTION CONTROL MANUAL, AIIMS, New Delhi, India.
10. INTEGRAL ASEPSIS FORUM (1975): Detergency and Its Role in Disinfection, Corlstadt N.J., *Airwick Industries Inc.*
11. CORLSTADT N.J. (1975): Technical Data in Hospital Comparative Testing of Disinfectant/Detergents: Integral Aspects forum; *Airwick Industries Inc.*
12. KELSEY J.C. AND MAURER I.M. (1974): An Unproved Kelsey – Skyes Test for Disinfectants, *Pharmaceutical Journal*, p. 213, 528.
13. KURTH J.M. (1984): St. Francis Hospital Environmental Services Policy Manual, *Aspen Publication*.

14. LOWBURY E.J.L., GEDDES A.M. AND WILLIAM J.D. (1998): Control of Hospital Infection- A Practical Handbook; 3rd Edition, *Chapman and Hall Medical*, London, Chapters 5 and 6.
15. MAURER ISOBEL M. (1985): Hospital Hygeine; 3rd Edition, *Richard Clay (The Chaucer Pren) Ltd.*, Great Britain.
16. RUTALA W.A. (1990): APIC Guidelines for Selection and Use of Disinfectants, *Am J. of Infection Control*, p. 18, 99.
17. SATPATHY S. (1996): Qualitative Evaluation of the Use of Chemical and Detergents in Hospital Laundry at AIIMS; *MHA Thesis*.

A STUDY OF ADOLESCENTS IN A RURAL AREA

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ABSTRACT

The findings of the study revealed that mean heights and weights in different ages of boys and girls were better than ICMR standards. Personal hygiene was found good/satisfactory among most of the adolescent boys and girls, however, a few of them had poor personal hygiene. While analysing their ambition, it was observed that a total of 54 students interested to become doctors, 38 engineers, 56 teachers, 34 defence officers and the rest wanted to join family business and salaried jobs. As far as their role model was concerned, the study revealed that film heroes and heroines were role models for 72 students, cricketers for 29 students, national leaders for 18 students and for the rest of the students parents/teachers were the role models.

Only 33 boys and 31 girls knew about conception but their knowledge about contraception was poor, the study revealed. Also 28 boys and 24 girls had some knowledge about sexually transmitted diseases and AIDS.

Key-words: Adolescence, Height, Weight.

Adolescence, the second decade of life (10-19 years) is a period of rapid development when young people acquire new capacities and are faced with many new situations. The rapid growth that occurs in adolescence demands extra nutritional requirements. During this period, more than twenty per cent of the total growth in stature and up to fifty per cent of adult bone mass are achieved. Adolescent girls also need additional requirements of iron up to fifteen per cent to compensate the physiological loss¹. Adolescence is also a time of mental and psychological adjustment, a situation of being no longer a child, but not yet an adult either¹.

In India, it is estimated that adolescents (10-19 years) constitute 21.8 per cent of the population i.e. 207 million. Married adolescents comprise 20 per 1000 population. Data (1992-1993) reveal that 6 per cent of the urban and 21 per cent of rural women aged 15-19 years were married before the age of 15 years¹.

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Due to various social taboos, ignorance and misconceptions about sex and sexuality, conception and contraception, adolescents form a special vulnerable group. Not much attention has been paid to this group by the society². They need nutritional, social, psychological and emotional support. It is easier to educate and motivate them as they are receptive group, more ready to learn and adjust to the new changes.² In the present study, an effort has been made to study their growth, beliefs, emotions, ambitions and relations with parents/teachers in Indori village, situated at 41 km. away from Pune.

MATERIALS AND METHOD

The students of class VIII and IX of Pragati Vidya Mandir, Indori were selected for the study with the permission of the principal. The objective of the study was explained to them. Their height and weight were recorded and also medically examined. A pre-tested questionnaire was given to them and they were asked to answer the questions. A total of 230 students participated in the study. The principal investigator guided the medical interns to carry out the survey. The study was carried out in August 2000. For personal hygiene a scoring system was followed for taking regular bath, trimming of nails, cleaning of hair, washing hands before meals, cleanliness of clothes/foot wear, presence of lice and skin diseases.

FINDINGS

A total of 230 students, 97 boys and 133 girls, between the ages of 12 and 18 years participated in the study. The mean height and weight as per age of boys and girls has been shown in Table 1.

Mean Height and Weight as per Age: The mean height as per age of girls in different ages was higher in the present study as compared to ICMR standards but in case of boys it was not so. With reference to weight, the boys and girls of the present study had better score as compared to ICMR standards (Table 1).

TABLE 1
MEAN HEIGHT AND WEIGHT AS PER AGE OF BOYS AND GIRLS AS
COMPARED TO STANDARDS OF INDIAN COUNCIL OF MEDICAL
RESEARCH (ICMR)

Height in Cms. for boys				Height in Cms. For girls		
Age	N	Observed	ICMR	N	Observed	ICMR
12	2	142.5	138.1	3	144.64	138.3
13	22	146.25	144	45	146.25	144.5
14	40	150.94	150.3	48	150.03	148.1
15	18	155.77	156.6	19	150.59	150
16	11	158.3	160.2	14	152.5	151
17	2	152.5	162.4	2	154.5	152.1
18	2	165.83	163.5	2	153.8	151.6
Total	97			133		
Weight in Kgs. For boys				Weight in Kgs. For girls		
Age	N	Observed	ICMR	N	Observed	ICMR
12	2	33.5	27.9	3	31.5	29.8
13	22	33.8	31.3	45	34.61	33.8
14	40	39.05	35.2	48	40.75	37
15	18	40.66	39.9	19	41.5	39.1
16	11	43.11	43.6	14	42.7	41.1
17	2	44	45.6	2	43.5	42
18	2	48.3	47.4	2	44.8	40.8
Total	97			133		

*(Indian Council of Medical Research Growth and Physical development of Indian infants and children, T.R.S. No. 18, 1972).

Personal Hygiene: The personal hygiene of boys and girls has been given in Table 2. One boy (1.03 %) and six girls (4.51%) showed poor grading. Data presented in Table 2 further reveal that 60.82 per cent of boys had very good personal hygiene as compared to girls (39.09%).

TABLE 2
PERSONAL HYGIENE SCORE OF BOYS AND GIRLS

Sex	Grading				
	Very good	Good	Satisfactory	Poor	Total
Boys	59 (60.82%)	30(30.9%)	7(7.22%)	1(1.03%)	97(100)
Girls	52 (39.09%)	53(39.85%)	22(16.54%)	6(4.5%)	133(100)

On routine examination of 230 students, the problems noticed were: Wax in Ear (37.39%), Tonsillitis (13.48%), Pallor (6.52%), Dental Caries (5.22%), Skin Infection (1.3%) and Nasal Polyp (0.87 %).

The students were given treatment for all the ailments. Those students who suffered from dental caries and nasal polyp were referred to the Talegaon General Hospital.

Background Characteristics of Students

Education of Parents: Fathers 38 (16.52%) and mothers 76 (33.04%) were illiterate. Others were educated up to different levels i.e. from primary to graduate level. Only 9 fathers (3.91%) and 2 mothers (0.87%) were graduates. Parents numbering 74 (32.17%) were found addicted to tobacco chewing mainly.

Relationship with Parents/Siblings: The students' relationship with parents was mainly based on love and respect, as expressed by 214 students (93.04%). Sixteen students (6.96%) were afraid of their parents. Most of the students had cordial relationship with their siblings.

Atmosphere at Home: Peaceful atmosphere was found in 216 (93.2 %) households while rest 14 (6.08 %) had quarrelsome and tense atmosphere.

Pocket Money: Most of the students got money to spend as per their requirements. Boys spent on eatables and girls liked to spend money on articles like bindis/hairclip, etc.

Study and School Atmosphere: All except 2 boys (2.06%) liked the school. Attendance was regular, except 3 boys (3.09%). The majority of students 189 (82.17%) were studying between one and two hours at home and rest 41 students (17.83%) studied for more than three hours. They were friendly with each other except 8 (3.48%).

Relation with Teacher: Love and respect for teachers were shown by 194 (84.35 %) students. Rest 36 (15.65 %) students were scared of them. All students evaluated favourably their teachers in respect of teaching, behaviour and dress.

Ambition and Role Model: Students who wanted to become doctors were 54 (23.48%), engineers 38 (16.5%) and teachers 56 (24.54%). 34 (14.78%) students wanted to join defence/police services and rest wanted to join family business/salaried jobs. 15 (6.52%) students did not express any opinion.

Film hero/heroines were role models for 72 (31.3%) students, cricketers for 29 (12.61) students, national leaders for 18 (7.83%) students and for the rest of the students, parents/teachers were the role models.

Mood Changes/Depression: Frequent loss of temper was felt by 45 male students (46.39%) and 74(55.64%) female students. Also change of mood was felt by 57 male students (58.76%) and 81 female students (60.9%). Study further revealed that female students felt more depression, the difference was statistically significant. Most common cause of depression was feeling lonely (Table 3).

TABLE 3
FEELINGS OF DEPRESSION AMONG MALE AND FEMALE STUDENTS

Sex	Occasional feeling of depression	Normal feeling	Total
Male	13	84	97
Female	107	26	133
Total	120	110	230

$X^2 = 109.10$, highly significant (Tabulated value of $X^2 = 3.84$)

Secondary Sex Characters among Boys: Out of 97 boys, secondary sex characters were present in 22 (22.68%) boys in the form of appearance of axillary hair, pubic hair and beard/moustache. Change of voice was observed in 21 (21.65%) boys.

Menarche Among Girl Students: Out of 93 girls who gave history of menarche, 78 (83.87%) had regular periods 15 (16.13%) had irregular periods and pain during menstruation was felt by 58 (62.37%). Mean menstrual age was 13.5 years as has shown in Table 4.

TABLE 4
MENARCHE WITH SYMPTOMS AMONG GIRL STUDENTS

Age at menarche (Years)	No. of girls	Periodicity of menstruation		Pain abdomen during menstruation	
		Regular	Irregular	Present	Absent
12	12(12.9%)	11(91.6%)	1(8.4%)	7(58.33%)	5(41.67%)
13	36(38.71%)	30(83.3%)	6(16.4%)	20(55.55%)	16(44.46%)
14	35(37.63%)	31(88.5%)	4(11.43%)	24(68.57%)	11(31.43%)
15	8 (8.6%)	5(62.5%)	3(37.5%)	5(62.5%)	3(37.5%)
16	1 (1.07%)	1(100%)	-	1(100%)	-
17	1(1.07%)	-	1(100%)	1(100%)	-
Total	93 (100%)	78(83.87%)	15(16.13%)	58(62.37%)	35(37.63%)

Mean menstrual age 13.5 years

Knowledge about Conception/Contraception: Thirty three boys (34%) and 31 girls (23.3%) knew about conception but knowledge about contraception was very poor among them.

Knowledge about Reproductive Tract Infection: Twenty eight boys (29%) and 24 girls (18%) were aware some of the diseases spreading through sexual intercourse. AIDS was found to be most commonly known disease which spreads by sexual intercourse.

DISCUSSION

The average height and weight of boys and girls as per age were compared with I.C.M.R. level. The height and weight of boys and girls of the project school were higher than the ICMR standards. The findings suggest that

probably the students may have been fed properly by their parents. Also, nowadays, both boys and girls are conscious of their appearance.

Personal hygiene of majority of the boys was better as compared to girls. Only one boy (1.03%) and 6 girls (4.51%) were graded poor. Similar findings were reported by Majumdar and Ganguli³ in their study of adolescent girls in Pune.

Film hero/heroines, cricketers and political leaders were the role models for most of the adolescents in the present study. Of course, in the present day world they are popular figures and media project them widely. As a result, the adolescent boys and girls are easily influenced by them. Some of the adolescents, both boys and girls experienced loss of temper, mood changes and depression. Ghai⁴ stated in his book that adolescents both boys and girls were prone to experience a state of depression due to various reasons. Michael Gelder et.al⁵ also observed that in adolescence considerable physical, psychosexual and social changes do occur and they were usually accompanied by some emotional turmoil.

In the present study mean menstrual age of girls was 13.5 years. Sixty two per cent of girls complained of pain in the abdomen during their menstruation. Majumdar and Ganguli³ in their study of adolescent girls in Pune found average age of menarche as 14 years and pain in abdomen during menstruation in 68.3 per cent of girls. Similar findings were noted by K. Aggarwal et al⁶ in their study of rural area of Delhi – prevalence of dysmenorrhoea was 70.8 per cent among girls who reached menarche.

Dr. D.C. Dutta⁷ has mentioned that the first menstruation occurs between 11 and 15 years with a mean age of 13 years. It was found that the knowledge of conception and contraception was limited among adolescents. Jeejebhoy⁸ in his study also found that whatever knowledge they had was incomplete and confused. The knowledge about reproductive tract infection (R.T.I) was also found to be poor. Ethno-Vision Group of Researchers⁹ in their study of adolescents for source of imparting family life education in Pune recommended that the multiple strategy be adopted like peer-led education, through teachers and doctors as students might prefer specific sources for specific issues. For example; girl students might want to know more about contraception through books while boys might be comfortable discussing the issue of masturbation or nocturnal ejaculation with peers rather than with their fathers.

REFERENCES

1. W.H.O. (1998): Strategies for Adolescent Health and Development in South East Asia Region, WHO Regional Office for South East Asia, New Delhi, p. 1 and 15.
2. PARUTHI RENU AND DUTTA P.K. (2000): Adolescent Health: An Emerging Priority, Paper Presented During Joint Annual Conference of Indian Public Health Association, Indian Association of Epidemiologists and Indian Society for Malaria and other Communicable Diseases at Agra on 10-12th March, 2000.
3. MAJUMDAR R. AND GANGULI S.K. (2000): A Study of Adolescent Girls in Pune, *Health and Population – Perspectives and Issues*, NIHFW, 23(2), p. 95-104.

4. GHAI O.P., GUPTA PIYUSH AND PAUL V.K. (2000): *Essential Paediatrics*; p. 45.
5. MICHAEL GELDER, RICHARD, MAYOO AND JOHN GEDDES (1999): *Child and Adolescent Psychiatry; Psychiatry, Oxford University Press*, p. 397.
6. AGGARWAL K., KANNAN A.J., PURI A. AND SHARMA S., (1997): Dysmenorrhoea in Adolescent Girls in Rural Area of Delhi. A Community based Survey; *Indian Journal of Public Health*, Vol. XXXXI, (8), July-Sept., p. 84-85.
7. DUTTA D.C. (1997): Gynaecological Problems from Birth to Adolescence, *Textbook of Gynaecology*, Calcutta, p. 478-479.
8. JEEJEBHOY (1996): Adolescent Sexual Behaviour: A Review of the Evidence from India, *ICRW*, Working Paper No. (3), December.
9. APTE HEMANT, ATHWAL S. AND GANPULE A. et al (1999): Family Life Education, *Ethno-Vision*, Apte House, p. 25-26.

NGOs AND HEALTH INSURANCE SCHEMES IN INDIA**

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ABSTRACT

The quality of health care available to the poor population in India is unacceptable. Services available through public health care facilities, which are supposed to be free, are often charged for. Moreover, private health care services available to the population are of poor quality. This paper explores the factors associated with the long-term success of non-profit community based insurers in providing preventive and curative health care services to the community in India. Besides the experiences of NGO-based schemes in other developing countries, the paper discusses some of the key features of prospective schemes and various constraining factors leading to their failures.

Key-words: Health Insurance, Non-profit.

So many non-profit Non-Governmental Organisations (NGOs) operate in India to provide preventive and curative health care services to the people. A small number of those NGOs also offer pre-payment health insurance schemes. Such non-profit community based insurers may offer the best hope of providing high quality, affordable and sustainable health care to the poor. This paper explores the factors associated with the long-term success of such schemes. Therefore, it is hoped, by identifying the factors, other NGOs can initiate risk-sharing schemes amongst their target population.

The need for involving NGOs in health insurance schemes in India arises due to the following four factors:

Firstly, In India, private expenditure accounts for roughly two-thirds of total health care spending. Studies by Sunder and Duggal et al reveal that only 3 – 4 per cent of the total health care expenditure and only 9 – 13 per cent of hospital care expenditure of the Government of India is incurred on the poor¹⁻².

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A study of 1000 households in Karnataka State in India also found out that 6 to 11 per cent of the total annual income was spent on health care.³ In the present study members of lower castes were found to spend a higher proportion of their annual income on health. Almost all out-of-pocket spending was on curative rather than preventive care⁴.

Secondly, the quality of health care services available to the people in India are of poor standards. Services available through public health care facilities which are supposed to be free but charged quite often. The private health care services available to the population are unaffordable.

Thirdly, health insurance coverage in India was very limited particularly among those who work outside the formal sector. Most of the insurance schemes were in the form of social security. They were provided through the Central Government Health Scheme (CGHS) and Employees State Insurance Scheme (ESIS). CGHS is a contributory health scheme that provides comprehensive medical care to the central government employees and their dependents. ESIS was an insurance system which provides both cash and medical benefits to the poor factory workers and their dependents. Another 1.8 million people were covered by the private insurance sold through the semi-autonomous Government Insurance Company (GIC)⁵.

Finally, government's combined expenditures, at the national, State and municipal levels accounted for only one-fifths of all health care spending in India. Most government funds are used to provide services directly through public hospitals, clinics and programmes. A disproportionate amount of government spending was on curative services in urban centers. This allocative inefficiency was unlikely to be corrected.

Experiences in Developing Countries

NGOs were playing an important role in health care provision in countries such as Zimbabwe, Tanzania, Uganda, Nepal, Mexico, Malawi and Ghana⁶.

Donald S. Shepard et al⁷ evaluated the design, management and operational efficiency of four health insurance schemes for informal sector in both rural and urban areas of Zaire region in sub-Saharan Africa. The study findings revealed that the insurance scheme has helped the people to have access to health care services in rural and urban areas. The authors, however, did not support the rapid implementation of a nation-wide conventional health insurance system as a feasible solution but suggested decentralised, locally managed plans for success. Further, the study also suggested to initiate different types of insurance schemes for out patient and inpatient care. For out-patient care, a system of pre-payment was suggested as a feasible method.

Andrew Creese and Sara Bennet⁸ reviewed the performance of thirty-six health insurance schemes for informal sector in developing countries. The schemes include health facility schemes generally initiated by hospitals, community schemes, cooperative schemes and the schemes run by the NGOs. The authors identified many problems in the existing schemes. Most of the schemes reviewed were suffering from limited population coverage, low cost recovery and limited ability to protect poorest member of the society. Moreover, many of the schemes had poor plan and design.

A review⁹ of experiences of varied groups of insurance schemes for informal sector in selected countries provided a number of insights as under:

- Most of the schemes are rural based and voluntary in nature.
- Government run and hospital based schemes explicitly cover both inpatient and outpatient services.
- Majority of the community based and NGO schemes cover only outpatient care.
- Most of the prepaid schemes have set a lower premium and provide limited services to the insurers.
- The coverage of target population was very low in most of the schemes with the exception of China, Korea and Japan.
- Experiences of community based schemes revealed that a committed, decentralised management contributes to the success of the schemes.
- Affordable premiums coupled with co-payment system could control the utilisation and cost of the insurance schemes.

NGOs and Health Care Services in India

As per estimates, NGOs provide health care to 5 per cent of the Indian population¹⁰. An important part of private health finance was the service provided by the voluntary and charitable organization of India. Berman (1992) notes while such groups do not account for a large share of health care, they were often the only source or only trusted source of health service to the population.

Many researchers have documented innovative and prepayment insurance schemes offered by some NGOs. The first thorough study of any such schemes appears to have been conducted in 1987 by the Indian Institute of Management (Ahmedabad) in preparation for a regional seminar organised by the Asian Development Bank¹¹. The private, non-profit health insurance schemes reviewed were the sewa-gram experience in Maharashtra and the Seba cooperative health society in West Bengal. Ford Foundation¹² reviewed the health financing experiences of four voluntary organisations in India. The study findings further indicated that voluntary health care programmes were funded by a number of sources, including government donor agencies, community and self generated funds and contribution made by the community as well as local efforts

made by the voluntary organisations to tap indigenous sources. The main sources were within the category of community and self-financing organisations which had many innovative financing mechanisms such as progressive fee scale, community based prepayment/insurance schemes and income generating schemes.

In follow-up of a study, twelve different voluntary organisations that provided health services were examined. Six out of twelve offer some form of prepayment or insurance scheme¹³. Other published literature makes a mention of various such schemes. The author also evaluated them on the basis of three criteria such as yield, equity and risk shared in prepayment/insurance schemes. The study showed that India's voluntary sector demonstrates much experimentation and innovation with a community and self financing methods including user charges, community based prepayment schemes, fund raising commercial schemes, etc.

Gupta et al¹⁴ examined the experience of voluntary health services (VHS) Madras, a non-profit organisation, in providing health services to low income people in urban areas. The study found that majority of the beneficiaries under medical aid plan of the VHS belong to very low monthly income group. The development of sliding scale of service and membership charge reflects its commitment to assuring access to low income patients in the locality. The coverage of the scheme was however limited and membership charges cover only a small per cent of recurrent costs.

In recent years, there have been some examples of private sectors establishing a tie up with government insurance companies working for financing health care. The Seba Cooperative health society in Calcutta, Apollo Hospital group in Hyderabad, Madras and Delhi, Batra Hospital in Delhi, Beach Candy Hospital in Bombay, Saurashtra Cooperative Hospital Society Bombay, Jamkher health project and Kasturba Hospital were few tie ups in this regard.

Couple of studies have also looked into the experiences of prepayment schemes run by NGOs in India. Pre-payment/insurance schemes were usually contributions made by individuals and households in advance for need based services. Only the sick availed of services. Therefore, in such financing schemes risks were shared between the healthy and sick. Schemes will provide different level of coverage for community and hospital care, varying from partial coverage to total coverage.

The review also revealed that there were only a few examples of NGOs operating in India and providing a wide range of health services. In fact India's NGO sector was one of the most developed in the region, though they were more active in the areas of preventive and promotive health care, their contribution to curative health care was also more substantial. The fact that several of these NGOs run dispensaries, clinics and hospitals which indicated that there is a lot of

scope to encourage and expand the role of NGOs in provision of curative health care. The Government has realized early that NGOs could be complementary to provide health care services. One of the encouraging facts of this movement has been the cooperation and helps extended to many NGOs by the government.

The government promotes NGOs in health sector for two main reasons: (i) to train its functionaries and (ii) to implement its programme in health care delivery. The Child In Need Institute (CINI), Kolkata; and SEWA are good examples of such cooperation. An attempt has been made here to review the working of some of the NGO based health insurance schemes in India and is given at Table 1. The review is based on published literature^{8, 15}. In the Table 1, for each private non-profit insurance scheme, the following information has been provided:

- (i) name, location and year of initiation;
- (ii) size of enrolled population;
- (iii) voluntary vs. mandatory;
- (iv) premiums;
- (v) benefits - direct vs. indirect;
- (vi) success and failure; and
- (vii) factors cited as underlying success/failure.

The purpose of this exercise is to provide examples of criteria based on which schemes have been evaluated and factors cited as contributory to success or failure.

Lessons Learnt and Prospective Schemes

Some of the key features for prospective schemes emerging from the lessons learnt from the existing schemes are as follows:

(i) Objectives or Purpose of Prospective Schemes

- Protecting poor population.
- Adequate coverage of target population.
- Low cost and essential care mostly consisting of primary care.
- An access is affected by geographical proximity and proper location of the facility.
- Mobilisation of funds with targeted exclusion of the poor.
- Focus on consideration of equity and efficiency in provision of services.
- Element of cost recovery with protection for the poor.
- Consumer satisfaction through improvements in quality and availability of care.

(ii) Risk Factors

- Mechanisms to prevent moral hazard in the prospective scheme.
- Adverse selection:- an option to counter adverse selection - designing of scheme where it was compulsory for all members to join. This will also check under – enrollment of the target population.
- The scheme should cover people of all ages and also the family members in case of working population. This would further ensure that insurance does not cover only healthy people but women and old people as well.
- The management funds should be handled professionally and suitable measures to be taken to guard against corruption and fraud.

(iii) Constraining Factors

- The premium should be based either flat rate or community rated premium. These should not be progressive.
- Since it was difficult to collect premiums from those not interested in joining the scheme, premium may be collected from source of wages payment.
- It seems that external support from government donors was almost a necessary condition to sustain the schemes.

(iv) Intervention for the Implementation

- Make the scheme more attractive for every one in the community to join.
- Proper monitoring of the scheme
- Well-defined benefit packages.

CONCLUSION

The NGO sector is all set to play a vital role in financing health care through health insurance schemes. This will also lead to a change in the system of health care delivery and payment mechanisms. In such a situation, the interests of the poor and under privileged sections of the society need to be guarded by appropriate regulatory mechanisms so as to ensure that equity and efficiency are maintained in the provision of health care.

TABLE 1
AN INVENTORY OF NON-GOVERNMENTAL, NON-PROFIT HEALTH INSURERS IN INDIA

Name, Location & year of initiation	Size of enrolled population	Voluntary Vs Mandatory	Premiums	Benefits (Direct Vs Indirect)	Success & Failure	Factors cited as underlying success/failure
1. Aga Khan Health Services (AKHS), Sidhpur, Gujarat (1996) (Meloj Milk Cooperative)	450	Has both mandatory & voluntary schemes	Premium varies from Rs.100 to Rs. 2500/- depending up on services.	Benefits include free outpatient consultation, discounted drugs and diagnostic services. Direct delivery.	Positive impact on utilization and equity. In some cases, it was not so.	Successful due to mandatory enrollment. Progressive fee schedule, Transparency in premium calculations. Low administrative costs, Favourable political foundations. Smooth functioning
2. Action for Community Organization Rehabilitation and Development (ACCORD), Nilgiris, Tamil Nadu (1991)	7000	Voluntary enrollment	Rs. 12 per person per year covers all illness requiring hospitalisation.	Direct delivery	Difficult to collect premium Low cost recovery.	Strong community group. Easy settlement of premium.
3. Appolo Hospital Assocation Madras, Tamil Nadu (1986)	10000	Voluntary enrollment	Annual premium for a family of 4 is Rs. 999, covers household costs of hospitalisation up to Rs. 17600.	Direct delivery	Evidence of ex-post moral hazard. Low consumer satisfaction. Declining enrollment.	Failure to cover outpatient expenses and chronic illnesses. Delays in processing claims.
4. Barpali Village Scheme, Orissa (1953)	Discontinued in 1961	Voluntary enrollment	Premium of 0.40 US \$ per family per year (1982 data)	Direct delivery	Reluctance to renew membership	Inadequate community participation. Isolation from traditional and political leaders.

5. Breach Candy Hospital, Bombay.	Corporate clients	Voluntary enrollment	Rs. 30 per month for a wide range of health services.	-	-	-
6. Goalpara, Shantiniketan, Rural West Bengal (1984)	1247	Voluntary enrollment	Premium of Rs. 18 either in cash or in kind.	-	-	Drug fee varied according to the economic conditions.
7. Mallur Milk Cooperative, Karnataka (1973)	7000	Mandatory enrollment	Premiums are paid from endowment fund.	Direct delivery	Cost recovery, entire health care expenditure of the community is now borne out of interest on the endowment fund Equity-difficult for poorest people in the area to join the scheme.	Strong economic base of the community; government provided vaccines, vitamins, contraceptives, disease surveillance, etc. Certain segments were not included.
8. Medinova Health Card Scheme, Calcutta .	35000	Voluntary enrollment	-	Direct delivery		-
9. Raigarh, Ambikapur Health Association, Raigarh, Orissa (1974)	75000	Voluntary enrollment	Premiums in kind	Direct Delivery	-	New members must wait for 2 months before they are entitled to benefits.
10. Saheed Shibsankar Sabha Samiti (SSSS) Burdwan, West Bengal (1978).	6800	Voluntary enrollment	Rs. 2/- per individual per year for the poor and Rs. 5/- per individual/yr for others	Direct delivery	-	Strict exclusion of non-members from benefits. .

11. Seba Cooperative Health Society (with GIC), Calcutta, West Bengal (1982)	<3000 families	Voluntary enrollment	Rs. 105 per member per annum	Direct delivery	Cost ineffective Limited cost recovery	Long hospital stay.
12. Self Employed Women's Association (with GIC) Ahmedabad, Gujarat (1992)	15000	Voluntary enrollment	Annual premium of Rs. 15/- per person	Direct delivery	In 50 per cent of cases requiring medical care, the benefits were sufficient. Equity-access to services and filing of claims was more difficult for rural women.	Strong referral network; ceiling on the amount of expenditures as guard against moral hazard, premiums can be directly deducted from member's bank account. Delays in processing claims; failure to cover families; slow and complex processing of claims.
13. Sewagram Kasturba Hospital, Wardha, Maharashtra (1972)	14,390	Voluntary enrollment	Premiums in kind	Direct delivery	Social benefits include stimulation of self-confidence, organisational ability, and development activities. Emphasis on low cost, preventive and promotive activities. Cost recovery is low.	High quality services; Trust of providers; charisma of organizer; premiums are in-kind, collected at convenient time.
14. Social Work and Research Centre (SWRC), Ajmer, Rajasthan (1972)	20,000	Mandatory enrollment	Premium of 2.85 US \$ per family per year	Direct delivery	Cost recovery is low	Programme emphasised on agricultural modernisation and general improvement in living standard. Committees in some villages fail to collect

						premium
15. Students Health Home, Calcutta, West Bengal (1955)	10,20,000	Voluntary enrollment..	Premium of Rs. 4 per annum, collected through the schools	Direct delivery	Extremely affordable premiums. Cost recovery members hip fee cover 34 per cent of total costs rising deficit, and falling quality of services. Inefficient spending.	Physician services were voluntary; some funds were kept/spent at the regional level; strict exclusion of non-members from benefits. Too much spent on inpatient services.
16. Tribhuvandas Foundation, Anand, Rural Gujarat (1993-94)	16 to 20 per cent of the target population of 800,000	Voluntary enrollment	(Primary care is free to all)	Indirect delivery	-	-
17. Voluntary Health Services (VHS), Medical Aid Plan, Chennai, Tamil Nadu(1963)	1,24,715	Voluntary enrollment.	Membership fee graded according to monthly income.	Direct delivery.	Extreme problem with adverse selection. Low level of cost recovery.	Many enroll only when referral treatment was required.

REFERENCES

1. SUNDER RAMAMANI (1992): Household Survey of Medical Care, *Margin*, 24 (2), January-March, p. 169-70.
2. DUGGAL R. AND SUCHETA A. (1989): Cost of Health Care – A Study in an Indian District, *FRCH*, Bombay.
3. MATHIYAZHAGAN K. (1998): Private-Public Mix in Health Development: An Empirical Estimate for Rural India, Bangalore; *Institute for Social and Economic Change*, p. 173.
4. SHARIFF ABUSALEH (1995): Health Transition in India; Working Paper No. 57, *National Council of Applied Economic Research*, New Delhi.
5. ELLIS R.P., GUPTA I. AND ALAM M. (1996): Health Insurance in India Prognosis and Prospects; *Institute of Economic Growth*, Delhi.
6. GILSON LUSY ET AL (1994): The Potential of Health Sector Non-Government Organizations: Policy Options; *Health Policy Planning*, 9(1), p.14–24.

7. SHEPARD S. DONALD et al (1990): Performance and Concept of Four Health Insurance Programmes in Rural and Urban Areas of Zaire, In: PAUL SHAW AND ANSWORTH, M, Financing Health Services Through User Fees and Insurance: Case Studies From Sub-Saharan Africa, World Bank Discussion Paper No. 294, World Bank.
8. CREESE A. AND BENNETT S. (1997): Rural Risk Sharing Strategies: Innovations in Health Care Financing; *Proceedings of a World Conference*, Washington D.C., World Bank, p. 163-82.
9. NAIR K. S. (1997): A Study on Possible Features of Potential Health Insurance Schemes for the Urban Informal Sector in Delhi; *A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Health Economics*, Chulalongkorn University, Bangkok.
10. HSIAO W.C. AND SEN P.D. (1995): Cooperative Financing for Health Care in Rural India; *International Workshop on Health Insurance*, Bangladesh.
11. INDIAN INSTITUTE OF MANAGEMENT (1987): Study of Health Care Financing in India; Ahmedabad.
12. FORD FOUNDATION (1989): Set of Four Case Studies on Costs and Financing of Voluntary Agency Health Projects; New Delhi.
13. DAVE P. (1993): Community and Self-Financing in Voluntary Health Programmes in India; *Health Policy and Planning*, 6(1).
14. GUPTA J.P. et al (1992): Financing of Health Care in Non-state Sector in India; *National Institute of Health and family Welfare*, New Delhi.
15. RANSON M. KENT (1999): The Consequences of Health Insurance for the Informal Sector: Three Non-Governmental, Non-Profit Schemes in Gujarat - Upgrading Document.

ROLE OF MEIOTIC MATURATION REGULATORY FACTORS IN DEVELOPMENTAL COMPETENCE OF MAMMALIAN OOCYTES

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ABSTRACT

In-vitro fertilization/embryo transfer (IVF/ET) is one of the important programmes in the field of assisted reproduction. Unfortunately, the success rate of IVF/ET is very poor. One of the important factors responsible for this is the retrieval of immature oocytes along with mature oocytes after superovulation induction. Maturation of these immature oocytes and the use of only meiotic competent oocytes for IVF could improve the success rate of this programme. It is also a very attractive treatment strategy for the establishment of pregnancy in infertile woman especially for polycystic ovarian syndrome (PCOS) patients. Undoubtedly, a significant improvement can be made by proper understanding of various factors, which are responsible for the complete maturation (nuclear and cytoplasmic maturation) of oocytes. Therefore, in the present article, the author has made an attempt to provide an up-to-date information on the biochemical and molecular changes that occur during meiotic maturation of mammalian oocytes.

Key-words: Mammalian oocytes, Follicles, Meiotic maturation.

Meiotic Competency of Oocyte and their Follicle Size

The maturing oocytes of mammals get meiotically arrested twice in its developmental stages. The first meiotic arrest is at diplotene stage of prophase first which may last from birth to puberty. The diplotene-arrested oocytes resume meiosis (in adulthood till menopause) and are arrested again at metaphase-II (M-II) after ovulation. The diplotene- arrested oocytes are encircled with several layers of cumulus cells (generally called dictyate stage oocytes) having germinal vesicle in the centre. Oocytes from early preantral follicles (growing follicles), which are not meiotically competent and do not undergo spontaneous maturation, if isolated and cultured in-vitro¹. Late preantral oocytes start acquiring meiotic competency and early antral follicles bear meiotically competent oocytes

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in mice, pigs, goats and cattle^{1,2,3}. In rodents, oocytes collected from preantral follicles do not undergo spontaneous maturation in-vitro^{1,4}. The nuclear maturation, cytoplasmic maturation and subsequent developmental competency of an oocyte are follicular size dependent in mouse⁵. Similarly, in non-rodent species, the ability of oocytes to resume meiosis is acquired if the follicle size reaches about 9-13 percent of its ovulatory diameter⁶. In goat, only 7 percent of oocytes were able to acquire meiotic competency, if isolated from preantral (<0.5-mm dia.) follicles. Oocytes isolated from late pre antral (0.5-0.8 mm dia.) follicles and cultured in-vitro, resumed meiosis but again arrested at M-I. All oocytes are meiotically competent, if isolated from early antral (<2.0 mm dia.) follicles⁷. Further, progressive increase in the maturational ability and developmental competency is acquired with the increase of follicle size in cow. Oocytes isolated from >3.0 mm follicles have poor rate of maturation and developmental competency compared to oocytes obtained from <6.0 mm follicles⁸. Monkey and human oocytes acquire meiotic competency at low rate as compared to other mammalian species. This could be because primate's oocytes acquire meiotic competency in the last phase of their follicular growth or that the meiotic maturation requires both stimulation and removal of inhibition⁹. This suggests that an increase in the maturational status and developmental competency of oocytes is associated with the increasing follicular size during late preantral to early antral stages. This may not be strictly correlated with the formation of antrum which occurs in the later stage of follicles^{1, 10}.

Cumulus Cells-oocyte Communication and Meiotic Maturation

Cumulus-intact oocytes are maintained at germinal vesicle (GV) stage by delicate cumulus cells oocyte connections. Corona radiata cells, part of the cumulus cells, penetrate through the zona pellucida and communicate with oocyte via gap junctions¹¹. The gap junctions are formed by extension of cytoplasmic processes of cumulus cells through zona pellucida¹². These gap junctions are composed of extracellular matrix protein belonging to connexin family¹³. Deficiency of these connexin molecule (Cx-37) does not allow follicles to mature and subsequently to ovulate¹⁴. The involvement of connexin-43 (Cx-43) in the cumulus cells-oocyte communication during resumption of meiosis has also been documented for bovine cumulus-oocyte complex¹⁵. The communication between cumulus cells and oocytes allows metabolic transfer and plays an important role in the regulation of meiotic maturation¹⁶. With the progress of meiotic competency, number of gap junctions and ionic coupling are decreased significantly^{2,16}. Thereafter, surrounding cumulus cells and oocyte are no longer coupled because of mucification and cumulus expansion¹⁷. It is also evident from our laboratory work that the cumulus cells dispersion is associated with acquisition of meiotic competency of rat oocytes⁴. During cumulus cells dispersion, the innermost layers of cumulus cells processes embedded in the zona pellucida and are retracted away from the surface of oocyte¹⁷. This is supported by the fact that if oocytes are retrieved from preovulatory follicles and cultured in-vitro in appropriate medium, they do undergo resumption of meiosis¹⁸. Removal of

cumulus cells from preovulatory oocytes shortens the period of spontaneous maturation indicating that the meiosis inhibitory signals are communicated to the oocytes via associated cumulus cells^{4, 18,19}. Associated cumulus cells maintain the meiotic arrest by transfer of meiosis inhibitory signals to oocytes through gap junctions. They also potentiate the fertilizability and developmental competency by synthesis and secretion of protein(s) required for cytoplasmic maturation of oocytes¹⁸. The removal of cumulus cells from preovulatory oocytes could mimic the actions of gonadotropins on meiotic maturation by interruption in the communication between cumulus cells and oocyte. This suggests the loss of gap junctions within the cumulus cells induce resumption of meiosis in rodents⁴. Removal of cumulus cells does not necessarily influence germinal vesicle breakdown (GVBD), rather, decrease final maturation and developmental competency of porcine oocytes in -vitro⁷.

The factors secreted by cumulus cells regulate the disruption of gap junctions, cumulus cells dispersion and subsequently controls meiotic maturation. In contrast, the loss of gap junctions within cumulus cells triggers the resumption of meiosis in pig cumulus oocyte complexes²⁰. A close association between cumulus cells and oocytes has been shown to be important in the production of competent mammalian oocytes matured in-vitro⁷. Some substance(s) synthesized by cumulus cells and transferred through the dynamic contacts by transzonal cumulus cell projections may actively modulate the maturation of cumulus-intact oocytes. This is supported by the findings that cumulus-enclosed and denuded oocytes matured in-vitro are morphologically alike but denuded oocytes have poor developmental competency after in-vitro fertilization (IVF)²¹. Taken together, these findings support the hypothesis that cell-to-cell communication among cumulus cells or between cumulus cells and oocytes maintain GV-stage in cumulus-enclosed oocytes. The removal of cumulus cells from preovulatory oocytes or gonadotropic surge reduces the flow of inhibitory signals by disruption of cumulus cells oocytes communication. On the other hand, certain factors produced by cumulus cells during maturation are essential for the oocyte to acquire fertilizability to sustain normal embryo development. Although, few studies have been carried out to explain the role of cumulus cells in the regulation of meiotic maturation but more studies are required to understand the role of connexin molecules in the gap junction formation and the precise mechanisms for communication between somatic cells and oocytes.

Role of Gonadotropins

LH is the primarily gonadotropin responsible for resumption of meiosis in-vivo and FSH also possesses this ability. Both gonadotropins induce maturation in cumulus-enclosed but not denuded oocytes in most of the mammalian species^{1,3}. We have also observed in our laboratory that the LH induces oocyte maturation only in cumulus- enclosed oocytes in-vitro⁴. These findings demonstrate the non-availability of receptors for gonadotropins at the level of oocytes². In cumulus-enclosed oocytes, gonadotropins stimulate cumulus cells to

generate cAMP through adenylate cyclase system and this transient increase in the intracellular cAMP disrupts the gap junctions. Reduced number of gap junctions leading to cumulus cells dispersion that positively overcomes the meiotic arrest results in the induction of oocyte maturation⁷. The gonadotropin-induced meiotic maturation is also reported by following cAMP-PKA pathway²². Another possibility for gonadotropin to induce resumption of meiosis is diacylglycerol-PK pathway²³. Later Su and coworkers explored third possibility for its action through inositol triphosphate (IP3)-Ca-calmodulin pathway to induce resumption of meiosis²⁴. Gonadotropin also stimulate MAP Kinase activity and thereby synthesis and secretion of meiosis activating sterol (MAS), a precursor of cholesterol, from encircling follicular cells to induce resumption of meiosis in mouse²⁵. Whatever the pathway gonadotropins follow, the ultimate function is to modulate cAMP level and reduce gap junctions in the cumulus cells leading to a reduction in the cumulus cells communication. The reduction in the communication, therefore, overcomes the inhibitory effect and results in the induction of meiotic maturation. Similarly, when these cumulus cells are removed and denuded oocytes are cultured in-vitro, they undergo spontaneous maturation². During routine method for collection of oocytes by superovulation procedure in several mammalian species including human, multiple follicular growth is stimulated by exogenous administration of FSH and final phase of oocyte maturation by hCG. But the concentration of gonadotropin hormone and their sequence of exposure may be important to recover meiotically competent oocytes, which can further increase the fertilization rate and early embryonic development¹. These findings support the hypothesis that gonadotropins stimulate meiotic maturation of oocytes indirectly through modulating cAMP levels in cumulus cells. Changes in the cAMP levels then affect cumulus cells-oocyte communication and enhance synthesis of stimulatory factor(s) from associated somatic cells to induce both nuclear and cytoplasmic maturation of mammalian oocytes²⁶.

Role of Steroids

Gonadotropins in combination with steroids have shown to induce meiotic maturation in immature oocytes and develop into the normal offspring in most of the mammalian species¹. Progesterone, through its negative feedback can affect LH pulse frequency and thereby play an important role in oocyte maturation and early embryonic development. However, it remains unclear whether steroid hormones, such as estradiol and testosterone, are also involved in the regulation of meiotic maturation². Further, it is documented that steroid hormones potentiate the inhibitory effect of FSH on cumulus-enclosed oocytes in-vitro². Very high physiological concentrations of testosterone, progesterone, pregnenolone and other steroids inhibit resumption of meiosis in-vitro. Meiosis-activating sterol (MAS), a natural cholesterol-precursor, extracted from bovine testis and human follicular fluid (FF-MAS), is also involved in the induction of meiotic maturation²⁶. FF-MAS appears to improve oocyte quality by supporting microtubules assembly. The mechanism by which it induces meiotic maturation

in mouse is MAP- Kinase-dependent pathway²⁷. Although, MAS induces microtubules organization and cytoplasmic maturation of mouse oocytes, further studies are required to establish the role of sterol in the regulation of meiotic maturation of mammalian oocytes.

Role of Cyclic Nucleotide Phosphodiesterase (cAMP-PDE)

Gonadotropin-induced meiotic maturation or spontaneous maturation in cumulus –enclosed or denuded oocytes do not occur in the presence of inhibitors of cAMP-PDE such as 3-isobutyl 1-methyl xanthine (IBMX), theophylline, caffeine and pentoxifylline in-vitro². These results suggest that the possible site for action for these inhibitors may be oocyte cAMP-PDE. An increase in PDE activity could stimulate a drop in the oocyte cAMP level associated with resumption of meiosis²⁸. Although, cAMP-PDE is not altered during maturation of mouse oocyte in-vitro, observations that IBMX and theophylline inhibit both GVBD and associated decrease in the level of cAMP strengthen the conclusion that PDE inhibitors reversibly block GVBD by their action on oocyte-PDE²⁸. These results demonstrate that the involvement of cAMP-PDE in the regulation of oocyte maturation. In addition, evidences from non-mammalian species have also indicated that the maturation associated decrease in the level of cAMP is associated with increased activity of oocyte cAMP-PDE^{29,30}. The PDE inhibitors reversibly block oocyte maturation by inhibiting PDE activity leading to accumulation of oocyte cAMP levels³¹. From our laboratory, we have also demonstrated that the caffeine and pentoxifylline (another inhibitors of PDE) prevent resumption of meiosis both in cumulus-enclosed as well as denuded rat oocytes in-vitro⁴. Another possibility that the activation of PDE by calmodulin (CaM) could regulate maturation associated decrease in oocyte cAMP. This has prompted investigation of a potential role of CaM in meiotic maturation²⁰. Involvement of CaM in the regulation of meiotic maturation indicates the role of calcium in the induction of oocyte maturation². Inhibitors of transmembrane calcium transport inhibit resumption of meiosis in-vitro. Further, an increase in the extracellular calcium concentration enhances meiotic maturation indicating the involvement of Ca ion in the regulation of meiotic maturation through calmoduline-dependent pathway in mammals. Taken together, these findings demonstrate that the oocyte PDE activity might be involved in the initial decrease of cAMP during gonadotropin-induced meiotic maturation in mammalian oocytes.

Role of Adenylate Cyclase

The adenylate cyclase is a membrane bound enzyme that catalyses the conversion of cytosolic ATP to cAMP for most of the physiological activities in eukaryotic cells. Activators of this enzyme, such as forskoline and cholera toxin induce oocyte maturation in cumulus-enclosed but not in denuded oocytes in rodents². These findings clearly demonstrate the availability of adenylate cyclase system at cumulus cells levels and forskolin or cholera toxin mimic the action of gonadotropin through adenylate cyclase-cAMP-mediated response³². Further,

LH induces elevation of cAMP levels in associated cumulus cells during the initial hours of oocyte maturation both in-vivo and in-vitro³. This transient increase in the oocyte cAMP level seems to strictly depend on progressive desensitization of granulosa cells to LH³³. This is likely to happen as a result of the down regulation of LH receptors caused by the peak concentration of LH required to trigger oocyte maturation. Unlike rodents, an increased level of cAMP within the oocyte is documented at the time of resumption of meiosis in rabbits,³⁴ sheep³⁵ and pig³⁶ oocytes suggesting the existence of adenylate cyclase- cAMP system at the level of oocytes in higher mammalian species. Taken together, these findings support the hypothesis that whether it is cumulus cells or oocytes, gonadotropins exert its action through adenylate cyclase-cAMP-pathway to induce resumption of meiosis of mammalian oocytes.

Role of cAMP

Continuous exposure of membrane permeable analogous of cAMP such as db-cAMP or 8-bromo-cAMP prevents spontaneous maturation of cumulus-enclosed or denuded oocytes in-vitro⁴. Similarly, other chemical compounds that are known to elevate intracellular cAMP, such as phosphodiesterase inhibitors (IBMX, theophylline, caffeine and pentoxifylline), adenylate cyclase activator (Forskolin and Cholera Toxin) also inhibit resumption of meiosis in-vitro. In addition, gonadotropins induce meiotic maturation by elevating cAMP level in the cumulus cells³². This seems to present an apparent paradox. If an increased level of oocyte cAMP inhibits resumption of meiosis, how gonadotropin induces meiosis resumption by elevating cAMP level in the oocytes? suggesting the dual role of cAMP in the regulation of meiotic maturation³². In cumulus-enclosed GV stage oocytes, the continuous transfer of an inhibitory level of cAMP from cumulus cells to oocytes is responsible for the maintenance of meiotic arrest. Removal of cumulus cells from their oocyte disrupts the flow of cAMP leading to the resumption of meiosis³². These studies suggest that the basal sustained level is responsible for the maintenance of meiotic arrest while transient increased level of cAMP, as in the case of LH- induced increased level of cAMP, induces oocyte maturation. Further, intracellular cAMP is involved in the regulation of cumulus cells expansion, cumulus cell-oocyte communication and developmental competency of oocytes³⁷. The above observations are supporting the hypothesis that cAMP is a key molecule in the regulation of oocyte maturation. Forskolin or gonadotropin-treatment stimulates transient increase in cAMP level in encircling follicular cells. These elevated cAMP levels in cumulus cells interrupt the communication with associated oocytes, leading in the reduced flow of cAMP to oocyte³². Another possibility for the decrease of oocyte cAMP level during the period of commitment to resume meiosis may be due to activation of cAMP-PDE since inhibitors of cAMP-PDE elevate cAMP level and prevent resumption of meiosis²⁸. On the other hand, in higher mammals such as in rabbit, sheep and pig oocytes, a higher concentration of cAMP throughout meiotic maturation maintains the meiotic arrest and a transient increase may facilitate resumption of meiosis³⁶. The transient increase in the concentration of cAMP may also exert

positive influence on the cytoplasmic maturation of cumulus-enclosed oocytes³¹. These findings potentiate the hypothesis that the cAMP is a key regulator for meiotic maturation in mammals.

Role of Calcium/Phospholipid-dependent Protein (PK-C) Kinase

Activators of calcium/phospholipid-dependent protein kinase (PK-C), such as phorbol esters and diacylglycerol prevent spontaneous maturation of oocytes probably by inhibiting phosphoprotein metabolism needed for meiotic maturation³. However, Calphostin C (a PK-C inhibitor) prevents resumption of meiosis in mouse³⁸. These findings clearly indicate the involvement of PK-C in the regulation of meiotic maturation. It is also evident that the PK-C is involved during M-I to M-II transition in mouse oocytes³⁹. PK-C activity appears resumption of meiosis and increases with respect to progress of meiotic maturation. The enzyme activity is to be highest at M-I arrested oocytes. Further, PK-C is associated with the regulatory mechanisms that are involved in delaying entry of oocytes into A-I³⁹. Therefore, loss of PK-C activity disrupts the critical M-I to M-II transition, leading to a precautious exit from meiosis. The stimulation of PK-C is sufficient and necessary event to induce meiotic maturation and that the mechanism by which a transient calcium burst trigger MPF activation involves PK-C-dependent pathway³⁸. Using an in vitro culture system, it is demonstrated that both inhibition of meiotic maturation and MAP kinase activation are mediated through PK-C-cAMP-dependent manner⁴⁰. These studies are in agreement that PK-C regulates the meiotic resumption by modulating both cAMP levels and MAP Kinase activity in mammals.

Mitogen Activated Protein (MAP) Kinase

Mitogen-activated protein kinase (MAP-K) is involved in many signal transduction pathways. It is a serine- threonine kinase and also known as extra cellular kinase (ERK). MAP kinase is activated during meiotic maturation in most of the mammalian species including human¹. The inactive enzyme gets phosphorylated to become active around the time of GVBD and the activity is maintained until M-II⁴¹. In rodents, MAP Kinase activation is associated with microtubule organization during meiotic maturation of mouse oocytes⁴². However, MAP kinase is not responsible for resumption of meiosis in rat oocytes⁴³. Further, MAP kinase activity is also required for the maintenance of MPF activity, spindle formation and M-II arrest in pig oocytes⁴⁴. In goat, MAP kinase activity is not associated with early events of meiotic events, but rather involve during post meiotic resumption events such as spindle formation and M-II arrest⁴⁵. In human, immature oocytes contain inactive MAP kinase, whereas the activity of the enzyme increases during meiotic maturation. The activity of enzyme decreases immediately after fertilization¹. Although the role of MAP kinase during meiotic maturation is not fully understood, it appears that MAP kinase is possibly involved during M-I to M-II transition, such as chromosomal separation, spindle elongation and formation of cleavage furrow in mammalian oocytes.

Role of MPF

Resumption of meiosis in dictyate stage oocytes of mammals accompanied by GVBD, chromosome condensation, spindle formation and emission of first polar body. These morphological changes are controlled directly or indirectly by maturation promoting factor (MPF), also called as M-phase promoting factor². MPF is a heterodimeric protein kinase consisting of p^{34cdc2} as a catalytic subunit and cyclin B as a regulatory subunit⁴⁶. Regulation of MPF activity depends upon the association of p^{34cdc2} kinase with cyclin B and the subsequent phosphorylation at tyrosin-15 and threonin-14 residues⁴⁷. The phosphorylation at these residues of p^{34cdc2} kinase after association with cyclin B is regulated by Myt-1 and Wee-1 kinases. MPF, in its phosphorylated form is inactive and called as pre-MPF. Pre-MPF is accumulated during G2 to M transition. Dephosphorylation at tyrosin-15 and threonin-14 residues of p^{34cdc2} kinase and subsequent association with cyclin B activate MPF⁴⁸. Further, cdc25 and nim-1 genes regulate dephosphorylation of pre-MPF to form active MPF. MPF is activated just prior to resumption of meiosis and increases until M-I and thereafter decreases during A-I to T-I transition. The activity again increases during M-II and is maintained until fertilization. The rephosphorylation of p^{34cdc2} kinase or cyclin B degradation through ubiquitin-dependent proteolysis is required for MPF inactivation⁴⁹. The periodic activation/ inactivation of MPF during meiotic maturation is the characteristic feature of mammalian oocytes.

Expression of Specific Genes during Meiotic Maturation

C-mos is a protooncogene encodes a serine-threonine protein kinase. Expression of this gene is involved in various aspect of oocyte maturation¹. C-mos regulate cyclin B activity through its phosphorylation and subsequently MPF activity of maturing oocytes. Therefore, it is involved in the maintenance of meiotic arrest at M- II in mammalian oocytes. C-mos is also involved for chromosome morphology and meiotic spindle formation in Porcine oocytes⁴². In human, it affects cyclin stability and thereby regulating meiotic maturation. The cdc- 25 and nim-1 gene regulate the dephosphorylation of threonine-14 and tyrosine-15 residues of a catalytic unit of MPF⁴⁶. The involvement of WEE- 1 gene in the regulation of MPF activity has also been documented. In addition, a few other genes are involved during periodic changes of activation/inactivation of MPF during meiotic maturation but their precise role during meiotic maturation is poorly understood. Therefore, the expression of specific genes and role of their products in the regulation of meiotic maturation require more studies to understand the molecular mechanism of final maturation in mammals.

CONCLUSION

Mammalian oocytes, arrested in the diplotene stage of first meiotic prophase, are regulated directly or indirectly by several factors. These factors include follicle size, cumulus cell communication, gonadotropins, steroids, cAMP,

cAMP-PDE, adenylate cyclase, PK-C, MAP-K, MPF and gene expression. Although, a number of studies has been carried out to clarify the role of these factors during meiotic maturation to some extent yet, more studies are required to understand the intricate mechanisms involved in the control of meiotic maturation oocytes. Any intervention in the maturation of oocyte in the follicle may affect fertilization and subsequently early embryonic development. Therefore, the assessment of meiotic competency of oocytes and use of only fully matured (nuclear as well as cytoplasmically matured) oocytes would be important for successful IVF and embryo transfer program. Clearly, improvement can be made to the maturation conditions for retention of the potential developmental competency of immature oocytes and this should be a priority for research on meiotic maturation. After all, it is meiotically competent oocytes that store all macromolecules to be utilized during fertilization and post fertilization events thereby decides the future of a developing embryo. Finally, understanding of biochemical and molecular mechanisms involved during meiotic maturation would not only improve embryo quality, it may also provide the potential role in female fertility regulation in human.

REFERENCES

1. TROUNSON A., ANDERIESZ C. AND JONES G (2001): Maturation of Human Oocytes in-vitro and their Developmental Competence; *Reproduction*, 121, p. 51-75.
2. KIKUCHI K., NAITO K., NOGUCHI J., SHIMADA A., KANEKO H., YAMASHITA M., AOKI F., TOJO H. AND TOYODA Y. (2000): Maturation/M- Phase Promoting Factor: A Regulator of Aging in Porcine Oocytes; *Biol. Reprod.*, 63, p. 715-722.
3. WASSARMAN P.M. AND ALBERTINI (1994): The Mammalian Ovum. The Physiology of Reproduction, 11nd Eds, Edited by KNOBIL E, AND NEIL JN; *Raven Press*, New York, Vol.-II, p. 79-122.
4. CHAUBE S.K., CHAKI S.P. AND MISRO M.M. (2000): Effects of Pentoxifylline and Caffeine on Spontaneous Maturation of Rat Oocytes. *Health and Population: Perspectives and Issues*, 23, p. 177-189.
5. SUN QY, LAI L., BONK A., PRATHER R.S. AND SCHATTEN H (2001): Cytoplasmic Changes in Relation to Nuclear Maturation and Early Embryo Developmental Potential of Porcine Oocytes: Effects of Gonadotropins, Cumulus Cells, Follicular Size and Protein Synthesis Inhibition; *Mol. Reprod. Dev.*, 59(2), p. 192-198.
6. GILCHRIST R.B., NAYDU P.L., NOWSHARI M.A. AND HODGES J.K. (1995): Meiotic Competency of Marmoset Monkey Oocytes is Related to Follicle Size and Oocyte-Somatic Cell Associations; *Biol. Reprod.*, 52, p.1234-1243.
7. CROZET N., DAHIREL M. AND GALL L. (2000): Meiotic Competency of In-vitro Grown Oocytes; *Jrnl. Reprod. Fertl.*, 118, p. 367-373.
8. BLONDIN P. AND SIRARD M.A. (1995): Oocyte and Follicular Morphology as Determining Characteristics for Developmental Competency in Bovine Oocytes; *Mol. Reprod. Dev.*, 41, p. 54-62.
9. DURINZI KL, SANGIA EM AND LANZENDORF SE (1995): The Relationship between Size and Maturation In-Vitro in the Unstimulated Human Oocyte; *Fertility Sterility*, 63, p. 404-406.
10. EPPIG J.J., WIGGLESWORTH K. AND OBRIEN M.J. (1992): Developmental Capacity of Mouse Oocyte Matured In-vitro: Effect of Gonadotropic Stimulation, Follicular Origin and Oocyte Size; *Jrnl. Reprod. Fertility*, 95, p. 119-127.

11. LAURINCIK T.A., KROSLAK P., HYTTEL P., PIVEKO J. AND SIROTKIN A.V. (1992): Bovine Cumulus Complex and Corona-Oocyte Disconnection during Culture In-vitro; *Reprod. Nutr. Dev.*, 32, p. 151-161.
12. WHITE T.W. AND PAUL D.L. (1999): Genetic Disease and Gene Knockouts Reveal diverse Connexin Functions; *Ann. Rev. Physiol.*, 61, p. 283-310.
13. KUMAR N.M. AND GILULA N.B. (1996): The Gap Junction Communication Channel; *Cell*, 84, p. 381-388.
14. SIMON A.M., GOODENOUGH D.A., LI E. AND PAUL D.L. (1997): Female Infertility in Mice Lacking Connexin 37; *Nature*, 385, p. 525-529.
15. VOZZI C., FORMENTON A., CHANSON A., SENN A., SAHLI R., SHAW P., NICOD P., GERMOND M. AND HAEFLIGER J.A. (2001): Involvement of Connexin 43 in Meiotic Maturation of Bovine Oocytes; *Reproduction*, 122, p. 619-628.
16. ZULKE K.A. AND BRACKETT B.G. (1990): Luteinizing Hormone Enhanced In-vitro Maturation of Bovine Oocyte with or without Protein Supplementation; *Biol. Reprod.* 43, p. 784-787.
17. SUZUKI H., JEONG B.S. AND YANG X. (2000): Dynamic Change of Cumulus –Oocyte Cell Communication During In-vitro Maturation of Porcine Oocytes; *Biol. Reprod.* 63, p. 723-729.
18. CHAUBE S.K., CHAKI S.P. AND MISRO M.M. (2000): Induction of Maturation and Fertilizability of Rat Oocytes In-vitro: Role of Cumulus Cells and Human Chorionic Gonadotropin; *Mol. Cell Endocrinol.*, 164 (w-18, abstract).
19. ISOBE N. AND TERADA T. (1998): Involvement of Meiotic Resumption in the Disruption of the Gap Junctions between Cumulus Cells Attached to Pig Oocytes; *Jrnl. Reprod. Fertl.*, 113, p. 167-172.
20. ISOBE N. AND TERADA T. (2001): Effects of the Factor Inhibiting Germinal Vesicle Breakdown on the Disruption of Gap Junctions and Cumulus Expansion of Pig Cumulus –Oocyte Complexes Cultured In-vitro; *Reproduction*, 121, p. 249-257.
21. GESHI M., TAKENOUCHI N., YAMAUCHI N. AND NAGAI T. (2000): Effects of Sodium Pyruvate in Nonserum Maturation Medium on Maturation, Fertilization, and Subsequent Development of Bovine Oocytes with or without Cumulus Cells; *Biol. Reprod.* 63, p. 1730-1734.

22. DOWNS S.M. AND HUNZICKER-DUNN M. (1995): Differential Regulation of Oocyte Maturation and Cumulus Expansion in the Mouse Oocyte-Cumulus Cells Complex by Site-Selective Analog of Cyclic Adenosine Monophosphate; *Dev. Biol.* 172, p. 72-85.
23. SU Y.Q., XIA G.L., HE C., FU G.D. AND YANG C.R. (1998): Studies on the Effects of Gonadotropins on the Meiotic Maturation of Cumulus-enclosed Porcine Oocytes In-vitro; *Chin. J Appl. Physiol.*, 14, p. 264-267.
24. SU Y.Q., XIA G.L., BYSKOV A.G., FU G.D. AND YANG C.R. (1999): Protein Kinase C and Intracellular Calcium are Involved in Follicle Stimulating Hormone- Mediated Meiotic Resumption of Cumulus Cell-Enclosed Porcine Oocytes in Hypoxanthine- Supplemented Medium; *Mol. Reprod. Dev.*, 53(1), p. 51-58.
25. HE C.L., DAMIANI P., PARYS J.B. AND FISSORE R.A. (1997): Calcium, Calcium Release Receptor, and Meiotic Resumption in Bovine Oocytes; *Biol. Reprod.*, 57, p. 1245-1255.
26. HEGELE HARTUNG C., KUHNKE J., LESSL M., GRONDAHL C., OTTESEN C., BEIER H.M., EISNER S. AND EICHENLAUB RITTER U. (1999): Nuclear and Cytoplasmic Maturation of Mouse Oocytes after Treatment with Synthetic Meiosis Activating Sterol In-vitro; *Biol. Reprod.*, 61, p. 1362-1372.
27. FAERGE I., TERRY B., KALOUS J., WAHL P., LESSL M., OTTESEN J.L., HYTTTEL P. AND GRONDAHL C. (2001): Resumption of Meiosis Induced by Meiosis-Activating Sterol has Different Signal Transduction Pathway than Spontaneous Resumption of Meiosis in Denuded Mouse Oocytes Cultured In-vitro; *Biol. Reprod.*, 65, p. 1751-1758.
28. BORNSLAEGGER E., WILDE M. AND SCHULTZ R. (1984): Regulation of Mouse Oocyte Maturation: Involvement of Cyclic AMP Phosphodiesterase and Colmodulin; *Dev. Biol.*, 105, p. 277-281.
29. HAIDER S. AND CHAUBE S.K. (1997): In-Vitro Effects of Forskolin, IBMX, and Cyanoketone on Meiotic Maturation of Follicle-Enclosed Oocytes; *Comp. Biochem. Physiol.* 115C, p. 117-123.
30. HAIDER S. AND CHAUBE S.K. (1995): Changes in Total cAMP Levels during Oocyte Maturation in the Catfish, *Clarius Batrachus*; *Comp. Biochem. Physiol.*, 112A, p. 379-385.
31. CHAUBE S.K. AND HAIDER S. (1996): Evidence for the Stimulation of Cyclic AMP Phosphodiesterase in the Catfish, *Clarius Batrachus*

- Oocytes by 17 α , and 20 β - dihydroxy-4-pregnen-3-one; *Jrnl. Exp. Zool.*, 277, p. 166-170.
32. DEKEL N., GALIANI D. AND SHERIZLY.I. (1988): Dissociation between the Inhibitory and the Stimulatory Action of cAMP on Maturation of Rat Oocytes; *Mol. Cell. Endocrinol.*, 56, p. 115-121.
 33. MATTIOLI M., GALEATI G., BACCI M.L. AND BARBONI B. (1991): Changes in Maturation Inducing Activity in the Cytoplasm of Pig Oocytes throughout Maturation; *Mol. Reprod. Dev.*, 30, p. 119-125.
 34. YOSHIMURA Y., NAKAMURA Y., ODA T., ANDO M., UBUKATA Y., KARUBE M., KAYAMA N. AND YAMADA (1992): Induction of Meiotic Maturation of Follicle enclosed Oocytes of Rabbit by a Transient Increase Followed by an Abrupt Decrease in Cyclic AMP Concentration; *Jrnl. Reprod. Fertil.*, 93, p. 803-812.
 35. MOOR R.M. AND HESLOP J.P. (1981): Cyclic AMP in Mammalian Follicle Cells and Oocytes during Maturation; *Jrnl. Exp. Zool.*, 216, p. 205-209.
 36. MATTIOLI M., GALEATI G., BARBONI B. AND SEREN E. (1994): Concentration of Cyclic AMP during Maturation of Pig Oocytes In-vivo and In-vitro; *Jrnl. Reprod. Fertil.*, 100, p. 403-409.
 37. MODINA S., LUCIANO A.M., VASSENA R., BARALDI SCESI L., LAURIA A. AND GANDO S. (2001): Oocyte Developmental Competency after In-vitro Maturation Depends on the Persistence of Cumulus Oocyte Communication which are Linked to the Intracellular Concentration of cAMP; *Ital. J Anat Embryol.*, 106 (2 suppl 2), p. 241-248.
 38. COLONNA R., TATONE C., FRANCIONE A., ROSATI F., CALLAINI G., CORDA D. AND FRANCESCO D.L. (1997): Protein Kinase C is Required for the Disappearance of MPF upon Artificial Activation in Mouse Eggs; *Mol. Reprod. Dev.*, 48, p. 292-299.
 39. VIVEIROS M.M., HIRAO Y. AND EPPIG J.J. (2001): Evidence that Protein Kinase C (PK-C) Participates in the Meiosis-I to Meiosis-II Transition in Mouse Oocytes; *Dev. Biol.*, 235, p. 330-342.
 40. LU Q., SMITH G.D., CHEN D.Y., YANG Z., HAN Z.M., SCHATTEN H. AND SUN Q.Y. (2001): Phosphorylation of Mitogen Activated Protein Kinase is Regulated by Protein Kinase C, Cyclic Adenosine Monophosphate, and Protein Phosphatase Modulators during Meiosis Resumption; *Biol. Reprod.*, 64, p. 1444-1450.

41. GORDO A.C., HE C.L., SMITH S. AND FISSORE R.A. (2001): Mitogen Activated Protein Kinase Plays a Significant Role in Metaphase Arrest. Spindle Morphology, and Maintenance of Maturation Promoting Factor Activity in Bovine Oocytes; *Mol. Reprod. Dev.*, 59(1), p. 106-114.
42. VERLHAC M.H., KUBIAK J.Z., WEBER M., GERAUD G., COLLEDGE W.H., EVANS M.J. AND MARO B. (1996): Mos is Required for MAP Kinase Activation and is Involved in Microtubule Organization during Meiotic Maturation in the Mouse; *Development*, 122, p. 815-822.
43. LU Z., XIA G., BYSKOV A.G. AND ANDERSON C.Y. (2000): Effects of Amphotericin B and Ketoconazole on Mouse Oocyte Maturation: Implications on the Role of Meiosis-activating Sterol; *Mol. Cell. Endocrinol.*, 164 , p. 191-196.
44. LEE J., MIYANO T. AND MOOR R.M. (2000): Localization of Phosphorylated MAP Kinase during the Transition from Meiosis-I to Meiosis-II in Pig Oocytes., *Zygote*, 8(2), p. 119-125.
45. DEDIEU T., GALL L., CROZET N., SEVELLEC C. AND RUFFINI S. (1996): Mitogen Activated Protein Kinase Activity during In-vitro Goat Oocytes Maturation and the Acquisition of Meiotic Competency; *Mol. Reprod. Dev.*, 45, p. 351-358.
46. GAUTIER J., SOLOMAN M.J., BOOCHER R.N., BAZAN J.F. AND KIRSCHNER M.W. (1991): CDC25 is a Specific Tyrosine Phosphatase that Directly Activates p^{34cdc2}; *Cell*, 67, p. 197-211.
47. NORBURY C. AND NURSE P. (1992): Animal Cell Cycle and their Control; *Annu. Rev. Biochem.*, 48, p. 397-411.
48. TAIEB R., THIBIER C. AND JESSUS C. (1997): On Cyclins, Oocytes and Eggs; *Mol. Reprod. Dev.* 48, p. 397-411.
49. GLOTZER M. MURRAY A.W. AND KIRSCHNER M.W. (1991): Cyclin is Degraded by the Ubiquitin Pathway; *Nature*, 349, p. 132-138.