

ROLE OF MYCOPLASMA AND ANAEROBES IN UNEXPLAINED INFERTILITY

B.M. AGARWAL • SANDIYA AGARWAL • MUKUL CHANDRA • ARCHANA GUPTA

SUMMARY

Unexplained infertility contributes to 9-14% of all infertile couples in India. 200 cases of U.I. (120 cases of P.U.I. & 80 cases of S.U.I.) along with 50 cases (control group) were studied during 1991-1994. The U.U. was more frequently isolated in infertility cases (30.8% in P.U.I. & 35% in S.U.I.). The isolation rate of U.U. was also high in semen of infertile couples. It was 12.5% in cases of P.U.I. and 45% in S.U.I. The M.H. was isolated in 4% in P.U.I. and 15% in S.U.I.

After chemotherapy with Doxycycline, 18 cases conceived within a period of 18 months follow up. The possible role of the association of mycoplasma and anaerobic organism leading to infertility has been discussed.

(Unexplained infertility, UI; Primary unexplained infertility, PUI; Secondary unexplained infertility, SUI; Mycoplasma hominis, M.H; Ureaplasma urealyticum U.U.).

INTRODUCTION

Since the isolation of Mycoplasma in man (Dienes & Edsall, 1937), this prokaryotic cell has been considered as one of the etiological agent in non-gonococcal urethritis (Csonka et al, 1977), chronic pelvic inflammatory diseases (Agarwal et al, 1991),

spontaneous abortions (Gupta, 1984; Agarwal et al, 1994) and still births (Kundsinn et al, 1969). Though recognition of its presence in the semen of infertile male suggests it as an etiological agent (Toth et al, 1983), isolation from normal population raises the question mark on its pathogenicity (Louvois et al, 1974; Schowb et al, 1976; Agarwal et al, 1993). Therefore its role in infertility has yet to be well

Dept. of Microbiology and Obst. & Gyn., S.N. Medical College, Agra.

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established (Friberg, 1980). Its association with secondary organisms, particularly anaerobic ones has not been explored well.

The present study was carried out to isolate mycoplasma species in primary and secondary unexplained sterility and to explore its possible role as a causative agent in association with anaerobic organisms in infertility.

MATERIAL AND METHOD

The present study was carried out in the Department of Microbiology and Sterility Clinic of Department of Obstetrics and Gynaecology, S.N. Medical College and Hospital Agra, during the period January 1991 to November 1994 using a strict criteria of unexplained infertility on normal findings at complete, general, physical and systemic examination including semen analysis (W.H.O. 1980), cervical mucus examination, endometrial biopsy, hysterosalpingography, laparoscopy and ultrasonography. Cases with any positive abnormality have been excluded in the present study.

Semen and high vaginal swab were collected and immediately immersed in liquid media (PPLO broth Base, Hi-media M-267) supplemented with antibiotics and horse serum. After incubation for 7 days in 5% CO₂ atmosphere, a flocculation without turbidity indicated a positive culture, which was plated on semi-solid media, having pH 7.2 for M.H. and 6.0 for U.U., to identify type of strain. All primary and secondary isolation was done in duplicate.

Identification of the species was based on morphological typing, staining of colonies and their biochemical behavior. Giemsa

staining was performed on Agar blocks containing Mycoplasma colonies (Cruickshank et al, 1980).

The anaerobic and aerobic organisms were isolated according to the criteria laid down by Finegold et al 1986).

OBSERVATION

The present study comprises of 200 cases of sterility (120 cases of PUI and 80 cases of SUI). 50 cases of same age group with confirmed fertility were included as control group. The isolation of mycoplasma species have been depicted in Table I.

None of the cases have both species of mycoplasma. The U.U. was consistently high in primary and secondary sterility cases but was strikingly absent in control group. This difference was statistically highly significant ($P < 0.01$).

The microbial flora in high vaginal swab culture (study and control group) as well as in semen have been depicted in Table II.

In high vaginal swab, the poly microbial flora pattern was seen with predominance of anaerobic organisms in 84 cases (70%) in Sub Group 'A', 64 cases (80%) in Sub Group 'B' and 16 cases (32%) in Control Group.

Similarly in semen the polymicrobial flora was seen with predominance of anaerobic organisms in 74 cases (61.6%) in Sub Group 1A', 50 cases (62.5%) in Sub Group 'B' and 11 cases (22%) in Control Group.

The difference in association of anaerobes alone or in combination with aerobic organisms was statistically significant ($P < 0.01$) in high vaginal swab and semen.

Table I
Positive Isolates Of Mycoplasma Sps. In High Vaginal Swab and Semen

Study Group (Total 200 cases)		Mycoplasma hominis	Ureaplasma urealyticum	Statistical Value	
			Z	P	
Sub Group A					
Primary infertility 120 cases	H.V.S.	6 cases (5%)	37 cases (30.8%)	7.31	<0.01
	semen	5 cases (4%)	15 cases (12.5%)	4.14	<0.01
Sub Group B					
Secondary infertility 80 cases	H.V.S.	7 cases (8.8%)	28 cases (35%)	6.56	<0.01
	semen	12 cases (15%)	36 cases (45%)	8.09	<0.01
Control Group 50 cases	H.V.S.	8 cases (16%)	None		
	semen	5 cases (10%)	None		

Table II
Aerobic and Anaerobic organisms in High vaginal swab and semen.

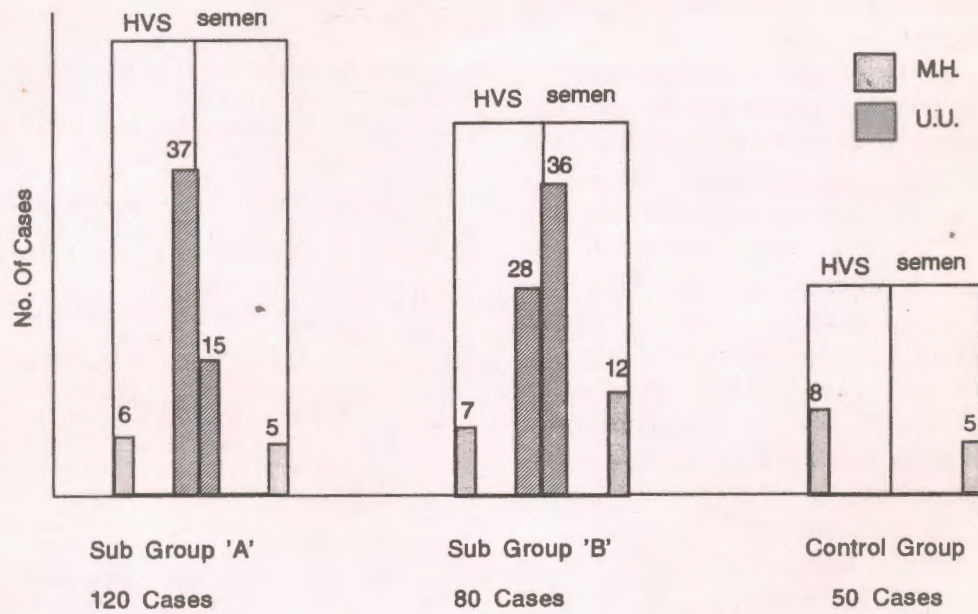
Study Group (Total 200 cases)		Aerobic organism	Anaerobic organism	Both	None
Sub Group 'A'					
Primary infertility 120 cases	HVS	20	28	56	16
	semen	28	52	22	18
Sub Group 'B'					
Secondary infertility 80 cases	HVS	12	34	30	4
	semen	18	34	16	12
Control Group 50 cases	HVS	04	10	06	30
	semen	07	08	03	32

RESULTS & DISCUSSION

Since the isolation of mycoplasma in female genital tract infections, it has been questioned as etiological agent, particularly in infertility (Gnarpe and Friberg J.

1972; Love et al, 1973; Upadhyaya et al, 1983; Horne 1986; Berclaz et al 1993).

The colonization of mycoplasma species in fallopian tube was initially considered as having no pathological effect



BAR Diagram illustrating comparison of M.H. & U.U. in High vaginal swab and semen in study and control group.

under light microscope (Taylor-Robinson and Carney 1974) but culture of the epithelial cells and electron microscopic studies have revealed the alteration in the morphology of epithelial cell (loss of cilia, swelling of epithelial cells) (Mardh et al, 1976). Further the release of metabolic products, ammonia in particular, had been held responsible for ultra microscopic changes which escape under light microscope.

The high incidence of anaerobic organisms particularly *Peptostreptococcus* sps. and *Bacteroid* sps. which produce urea splitting enzymes (Sutter and Finegold, 1985) and their association with *Mycoplasma* sps. in high vaginal swab and semen may reduce the functional activity ultimately leading

to infertility.

In the present study also, the incidence of *Ureaplasma urealyticum* was high in both primary and secondary infertile couples (30.8% and 35% in high vaginal swab and 12.5% and 45% in semen, respectively). The anaerobic organisms either alone or in combination with aerobic organism were seen in 70% in primary sterile and 80% in secondary sterile females. Similarly in semen, the incidence of anaerobic organism was also very high in primary sterility (61.6%) and secondary sterility (62.5%), and very low in control cases (22%).

The high incidence of *Ureaplasma urealyticum* in association with polymicrobial flora, in particular anaerobic organism, may release the metabolic products which may

damage the pathophysiology of the tubular epithelium without inducing any light microscopic alteration.

In the followup, 35 couples had UU both in HVS and semen and 46 cases had UU either in semen or HVS were treated with Doxycycline (Vibazine DT) with a dose of 200 mg. twice on the first day and 200 mg. once a day subsequently for 14 days, in study group only. The cycle was repeated after an interval of 1 month for 3 times. 6 cases were lost in the followup. A total of 18 cases (24.0%, 13 out of 35 and 5 out of 46) conceived after treatment, which showed that there is restoration of normal pathophysiology allowing the sperm to pass on from the tube.

Further, a bigger study is needed to support this hypothesis.

REFERENCES

1. Agarwal S., Agarwal B.M., Budhraj I.: *J. Obstet Gynecol Ind.* 41; 518, 1991.
2. Agarwal S., Agarwal B.M., Gupta K., Gupta B., Mudgal N.: *J. Obstet. Gynec. Ind.* 43; 791, 1993.
3. Agarwal S., Agarwal B.M., Gupta K., Ansari K.H.: *J. Obstet. Gynec. Ind.* 1994 (Under publication).
4. Berclaz G., Hanggi W., Birkhauser M., Gyr T., Konig C., Gerber-Huber S., Drescher H.: *Chlamydia and mycoplasma infections in men of couples with involuntary sterility. Geburtshilfe - Frauenheilkd;* 53 (8); 539, 1993.
5. Cruickshank R., Duguid J.P., Marmion B.P., Swain R.H.A.: *Med. Microbio.* 12; 527, 1980.
6. Csonka G.W., Taylor-Robinson D., Prentice M.J.: *Q. J. Med.* 46; 309, 1977.
7. Dienes L., Edsall G.: *Proc. Soc. Exp. Biol. Med.* 36; 740, 1937.
8. Finegold S.M., Baron Ellen J.O., Bailey and Scott's diagnostic microbiology, 7th ed., p500 1986.
9. Friberg J.: *Fertil. Steril.* 33; 351, 1980.
10. Gupta U.: *C.V. Soc. Mathura*, 27 Feb., 1984.
11. Gnarpe H. and Friberg J.: *Am. J. Obstet. Gynec.* 114; 727, 1972.
12. Horne H.W.: *Infertility and PID, JAMA;* 256; 591, 1986.
13. Kundsir R.B., Driscoll S.G., Horne H.W., Scott J.M.: *Fertil. Steril.* 20; 1017, 1969.
14. Louvois J., Blades M., Harrison R.F., Hurley R., Stanley V.C.: *Lancet;* 2; 1073, 1974.
15. Love W., Jones M., Andrews B., Thomas M.: *Lancet;* 1; 1130, 1973.
16. Mardh P.A., Westrom L., Von Mecklenburg C.: *Br. J. Vener. Dis.* 52; 52, 1976.
17. Schowb B.C., Jabobs Y.R., Hlyen E., Freedman P.: *S. Afr. Med. J.* 50; 445, 1976.
18. Sutter V.L. and Finegold S.M.: *Anaerobic Bacteriology Manual*, 1985. IVth ed.
19. Taylor-Robinson D., Carney F.E.: *Br. J. Vener. Dis.* 50; 212, 1974.
20. Toth A., Witkin S.S.: *Fertil. Steril.* 40; 805, 1983.
21. Upadhyaya M., Hibbard B.M., Walker S.M.: *Fertil. Steril.* 39; 814, 1983.
22. World Health Organisation; *Laboratory Manual for the examination of human semen Singapore, Press concern;* p7, 1980.