MYCOPLASMA INFECTION IN PREGNANT RATS: LOW VIABILITY OF FOETUSES AND NEWBORN OFFSPRING

Y. NAOT*, M. SHARF† AND A. KLEIN†

* Department of Immunology, Technion, School of Medicine, POB 9649, Haifa, Israel, and

† Department of Obstetrics and Gynaecology, Rothschild Hospital, Haifa, Israel

THE role of mycoplasmas in human reproductive failure is still controversial. This is because the risks associated with the infection of human volunteers make it necessary to rely on statistical comparisons of the incidence of mycoplasma infection in infertile couples with that in normal fertile couples. It is true that *Mycoplasma hominis* and T-strain mycoplasmas have been isolated from gestational products in instances in which birth was premature or newborn offspring were of low weight (Harwick *et al.*, 1967; Jones, 1967*a* and *b*; Klein, Buckland and Finland, 1969; Kundsinn and Driscoll, 1970; Braun *et al.*, 1971; Caspi, Solomon and Sompolinsky, 1972). On the other hand, De Louvois *et al.* (1974) found no statistical difference between the rates of mycoplasma infection in fertile and infertile couples.

In view of this controversy, an animal model was examined by Taylor-Robinson *et al.* (1975) who found that foetal wastage occurred as a direct consequence of *Mycoplasma pulmonis* infection in the mouse. Mice infected with *M. pulmonis* 9 days after mating showed an increase in foetal mortality.

In the present study, we have attempted to throw further light on the effects of mycoplasma infection both during gestation and in the neonatal period, and we discuss briefly a possible mechanism for mycoplasma pathogenicity.

The organism chosen for our study was *Mycoplasma arthritidis*, a murine mycoplasma that produces latent infections and becomes pathogenic under appropriate conditions of stress (Tully, 1969); it has been reported to induce polyarthritis, conjunctivitis and urethritis in experimentally infected mice and rats (Ward and Jones, 1962; Cole, Golightly-Rowland and Ward, 1976). It has been shown to be indistinguishable in serological, biochemical and pathological properties from a common organism, originally recovered from the human genital tract and classified as *M. hominis* type 2 (Lemcke, 1964; Tully, 1965; Cole *et al.*, 1976). Further studies have shown that *M. arthritidis* strains can also be isolated from human tissues, thus supporting the concept that they may be either commensal or pathogenic in man. This influenced our choice of organism.

MATERIALS AND METHODS

Animals. Male and female Charles River rats aged 2 months were used. These animals were bred and reared under specific pathogen-free conditions.

Mycoplasma strain. *M. arthritidis* strain PG-6 was kindly provided by Dr J. G. Tully (NIAID-NIH, Bethesda, Maryland, USA). Broth and agar media were prepared by a

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TABLE I

Comparison of the first (normal) pregnancy of control rats with the following pregnancy during which they were inoculated with sterile broth medium

Animal number	Observations on first pregnancy					Observations on second pregnancy				
	Duration of pregnancy (days)	Weight increase (%) of dam*	Total number of offspring	Number of offspring alive at birth	Number of offspring alive 10 days after birth	Duration of pregnancy (days)	Weight increase (%) of dam	Total number of offspring	Number of offspring alive at birth	Number of offspring alive 10 days after birth
1	23	151	5	5	5	21	149	6	6	5
2	22	164	10	10	10	22	159	13	12	12
3	22	153	13	13	13	21	134	15	15	15
4	21	147	10	10	10	21	127	11	10	9
5	22	150	11	11	11	21	141	12	12	12
6	21	151	12	12	12	21	141	12	12	12
7	22	144	10	10	10	22	145	10	10	10
8	22	141	9	9	9	22	143	8	8	8
9	22	149	10	10	9	22	147	10	10	9
10	23	151	5	5	4	21	142	6	6	5
11	22	164	10	10	10	22	148	13	13	12
Total	242	1665	105	105	103	236	1576	116	114	109
Mean	22	151.4	9.5	9.5	9.4	21.4	143.2	10.5	10.3	9.9

* Weight on 21st day of pregnancy expressed as a percentage of weight at the time of mating.

modification of Hayflick's method (1965) as described on an earlier occasion (Beck, Ginsburg and Naot, 1977). Cultures were incubated at 37°C for 3–4 days and the suspensions stored at –75°C; 0.1-ml samples of serial tenfold dilutions were plated on agar and counted after incubation for 7 days. The numbers of organisms were expressed as colony forming units (CFU) per ml.

Experimental procedure. The phases of oestrus of each female animal were observed over two reproductive cycles, vaginal smears being taken by means of a glass spatula sterilised with 70% ethyl alcohol and immersed in sterile normal saline. Then, on the appearance of cornified cells in a smear, each female was placed in a cage with a male and left for about 20 h. The presence of spermatozoa in a vaginal plug and their appearance in a vaginal smear were taken to indicate the onset of gestation. The presence of a plug without spermatozoa was considered inconclusive.

Advantage was taken of the first mating to eliminate infertile animals. Of a group of 45 females born on the same day five failed to conceive and were discarded. The remaining 40 were divided into two groups of 25 and 15. During their second pregnancy 7–11 days after conception, rats in the first group were inoculated intravenously with 1 ml of mycoplasma suspension containing 10^{10} CFU; rats in the second group were kept in a separate room and served as controls, being inoculated with 1 ml of sterile broth. Injections were administered under general anaesthesia with chloroform, maintained for about 2–3 min.

During the gestation period the animals were weighed daily or on alternate days, and examined for swollen joints daily, in order to estimate the progress of pregnancy and to detect arthritis. On the 20th day four or five animals in each group were killed and their ovaries, spleen, uterus and lungs, as well as foetuses and placentae (removed by caesarean section) were examined for mycoplasmas as described by Ganaway *et al.* (1973).

Sterile gloves were used when the litters were counted, and great care was taken to cause only a minimum of disturbance and so forestall devouring of the offspring. Offspring were examined for viability immediately after birth and subsequently every few hours for 10 days. Suckling was allowed for 21 days before the offspring were removed and the mothers rested for 14 days.

RESULTS

Tables I and II list the observations for the control group (11 rats) and experimental group (20 rats) respectively, and draw a comparison between the first (normal) and second (manipulated) pregnancies. Table III, derived from the data in tables I and II, summarises information on the effects of the mycoplasma infection.

Experimental and control rats in their first (normal) pregnancy

No significant differences occurred between the two groups in terms of mean gestation period, mean litter size, percentage of offspring alive at birth, and percentage of offspring alive 10 days after birth.

Control rats in their first (normal) and second (manipulated) pregnancies

No significant differences occurred between the two pregnancies in terms of mean gestation period, mean litter size, percentage of offspring alive at birth, and percentage of offspring alive 10 days after birth. In other words, inoculation of rats with broth 7–11 days after conception did not affect reproduction. However, in this study, as in a previous study (Klein *et al.*, 1978), the inoculation of rats with broth 7–11 days after conception influenced to some extent the

TABLE II
Comparison of the first (normal) pregnancy of experimental rats with the following pregnancy, during which they were inoculated with mycoplasmas

Animal number	Observations on first pregnancy					Observations on second pregnancy				
	Duration of pregnancy (days)	Weight increase (%) of dam*	Total number of offspring	Number of offspring alive at birth	Number of offspring alive 10 days after birth	Duration of pregnancy (days)	Weight increase (%) of dam*	Total number of offspring	Number of offspring alive at birth	Number of offspring alive 10 days after birth
12	22	160	12	12	12	21	129	4	2	1
13	22	154	10	10	10	22	128	2	1	0
14	23	161	8	8	8	22	126	11	5	2
15	21	149	10	10	10	22	149	11	11	8
16	22	160	13	13	13	21	127	4	2	0
17	22	165	10	10	10	22	148	9	7	4
18	22	164	13	13	12	22	127	5	3	1
19	23	159	10	10	10	22	121	5	3	1
20	23	163	13	13	13	22	132	9	7	6
21	22	152	11	11	11	22	140	11	9	9
22	22	159	15	15	15	21	134	14	10	7
23	22	171	17	17	16	21	123	14	8	5
24	22	160	10	10	10	21	137	11	8	5
25	22	149	9	9	9	22	125	4	3	0
26	22	ND†	11	11	10	21	ND†	9	9	3
27	22	152	10	10	10	20	120	10	8	5
28	22	153	7	7	7	22	164	10	7	4
29	21	166	12	11	11	22	136	12	11	7
30	22	154	10	10	10	22	144	9	1	1
31	22	153	11	11	11	22	140	10	5	5
Total	441	3004	222	221	218	432	2550	174	120	74
Mean	22.05	158.1	11.1	11.0	10.9	21.6	134.2	8.7	6	3.7

* Weight on 21st day of pregnancy expressed as a percentage of weight at the time of mating.

† ND = Not determined.

normal course of weight increase during pregnancy. This effect is attributed to the inoculation, or the accompanying anaesthesia, or both.

Experimental and control rats in their second (manipulated) pregnancies

Examination of the data reveals the effects of mycoplasma infection. Table III clearly shows that the percentage of offspring alive at birth was significantly ($P < 0.001$) higher in the control group than in the experimental group. The same was true of the percentage of offspring alive 10 days after birth. In addition, the mean litter size of the experimental group was less than that of the control group, and less than that of either group in the first (normal) pregnancy.

TABLE III

Data relevant to the effects of mycoplasma infection on reproduction, derived from control and experimental rats in their first (normal) and second (manipulated) pregnancies

Pregnancy	Number of rats	Treatment during pregnancy	Mean duration of pregnancy (days)	Mean total number of offspring	Mean percentage of offspring alive at birth	Mean percentage of offspring alive 10 days after birth
1st	11	None	22	9.5	100	98.1
	20	None	22.05	11.1	99.5	98.2
2nd	11	Broth injection	21.4	10.5	98.2	93.9
	20	Mycoplasma injection	21.6	8.7	68.9	42.5

This table is based on information given in tables I and II.

Microbiological and clinical examination of rats

No arthritis was seen in any animal. Of the animals killed for examination, all from the experimental group but none from the control group had foetuses and placentae that were infected with mycoplasmas. These findings confirm our previous report (Klein *et al.*, 1978).

DISCUSSION

Our results clearly demonstrate that mycoplasma infection has an adverse effect on pregnancy in rats. This effect was reflected in a significant decrease in size of litter, and reduced viability of offspring at birth and, still more, at 10 days after birth. The smaller weight increase observed in mycoplasma-infected pregnant rats than in control pregnant rats (see tables I and II) was compatible with the reduced number of foetuses. The decrease was attributable to foetal resorption.

Our results are in agreement with those of Taylor-Robinson *et al.* (1975) in showing that mycoplasma infection of pregnant rodents can affect foetal survival; they also show that survival of the newborn offspring can be affected.

On an earlier occasion we showed that *M. arthritidis* induced extensive non-specific lymphocyte transformation in rats (Naot, Tully and Ginsburg, 1977); this observation, together with the well-documented isolation of mycoplasma from foetal organs (Taylor-Robinson *et al.*, 1975; Klein *et al.*, 1978), raises the question of whether pathogenicity is associated with mitogenicity.

SUMMARY

The controversy regarding the possible role of genital mycoplasma infection in human reproductive failure gave rise to a study in which pregnant rats, previously shown to be of normal fertility, were inoculated 7–11 days after conception with either *Mycoplasma arthritidis* or an equal volume of sterile broth medium. Repeated observations during the course of pregnancy revealed several pathogenic effects.

A statistically significant decrease was observed in the mean litter size of infected mothers. Furthermore, the offspring showed low viability at birth and, still more, at 10 days after birth. Our data are compatible with the hypothesis that genital mycoplasma infection plays a role in reproductive failure.

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