INFECTION AND IMMUNITY, Oct. 1982, p. 368-370
0019-9567/82/100368-03$02.00/0

Vol. 38, No. 1

NOTES

Chlamydia trachomatis Infection of Mouse Trophoblasts

JOYCE BANKS,[1] ROBERT GLASS,[2,3] AKIKO I. SPINDLE,[3] AND JULIUS SCHACHTER[1]*

Departments of Laboratory Medicine[1] and Obstetrics and Gynecology[2] and Laboratory of Radiobiology and Environmental Health,[3] University of California, San Francisco, California 94143

Received 3 March 1982/Accepted 9 June 1982

Chlamydia trachomatis (strains L2 and DE) were found to infect fertilized mouse ova in vitro. Inclusions were found in the trophoblast, but not in the embryo.

Chlamydia trachomatis is a common sexually transmitted pathogen whose role is human infertility appears to be emerging (2, 5, 9). In females, the anatomical site usually infected is the cervix. In addition, *C. trachomatis* has been recovered from the fallopian tubes and endometria of women with acute salpingitis (8, 9). Infertility could result from tubal damage due to chronic infection or as a sequela of acute salpingitis. Other mechanisms could include infection within the uterus or of fertilized ova. There are no useful in vitro models to study the possible roles of *Chlamydia* in infertility. Studies from England have shown that *C. trachomatis* is capable of infecting human fallopian tube explants in vitro, but the infection does not appear to reduce ciliary action (3). We have confirmed this observation, finding a several-log increase in chlamydial titer in the absence of gross or histopathological damage to human fallopian tube organ cultures.

In an effort to determine whether *C. trachomatis* could infect fertilized ova, we used a murine system which allows collection of reasonable numbers of fertilized eggs. Infecting *C. trachomatis* strains used were an LGV (434/L2) and a recently isolated DE serotype.

Mouse blastocysts were flushed from the uteri of superovulated mice on day 4 of gestation (6). The blastocysts were collected in petri dishes containing modified Eagle basal medium (6) without antibiotics and supplemented with 20% fetal calf serum and 2% BSA-V (growth medium).

With the aid of a dissecting microscope, 4 to 12 blastocysts were drawn up into a fine-tipped Pasteur pipette and transferred onto 12-mm glass cover slips in a 1-dram vial containing 1 ml of growth medium. At 24 h later, the embryos were attached to the glass and observed to have an inner cell mass surrounded by a spreading outgrowth of trophoblast cells (approximately 30 to 60 cells) (Fig. 1). The cells continued to migrate from the central mass for 7 days. Because of the small number of embryos cultured on each cover slip and the distance between them, the individual outgrowths did not become continuous with the outgrowth from other embryos. The cultures were infected between 48 to 96 h after plating, and there was no significant difference in susceptibility.

The chlamydiae were then introduced by centrifugation (2,800 × *g*, 1 h) or by simply replacing the tissue culture maintenance medium for 1 h with medium containing 10^2 to 10^5 inclusion-forming units and then washed. The trophoblast cells were found to be susceptible to chlamydial infection (Fig. 2 and 3). Centrifugation was not required to establish infection. Approximately 20 to 30% of the trophoblast cells contained iodine- and Giemsa-staining chlamydial inclusions at 48 to 72 h postinfection with the DE serotype, and up to 90% of the cells had inclusions when the same inoculum size of the L2 serotype was introduced. These trophoblast cells were very large, and the inclusions were much larger than those seen in the McCoy or HeLa cells used for chlamydial isolation attempts. The inclusions were as large as 50 to 100 μm in diameter, a size which made it impossible to photograph the inclusion in a single photographic field at ×1,000. The inner cell mass measured approximately 100 to 220 μm; the trophoblast cells were usually 50 to 100 μm in diameter. It was not possible to quantitate the infection or the efficiency of the infection because there were not discrete monolayers; 4 to 12 fertilized ova were planted per cover slip. Infection was not observed in the inner cell mass but was found exclusively in trophoblast cells.

Although it is recognized that this is a murine

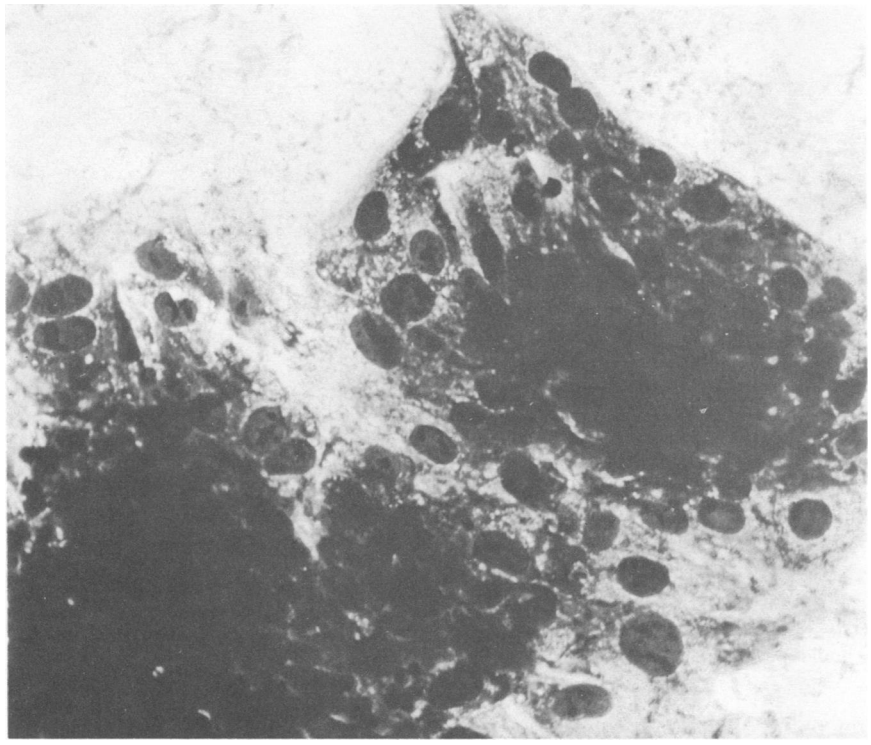

FIG. 1. Giemsa stain of uninfected fertile mouse ova after 5 days on glass. The dense inner cell mass represents the embryo, and the spreading monolayer is trophoblast.

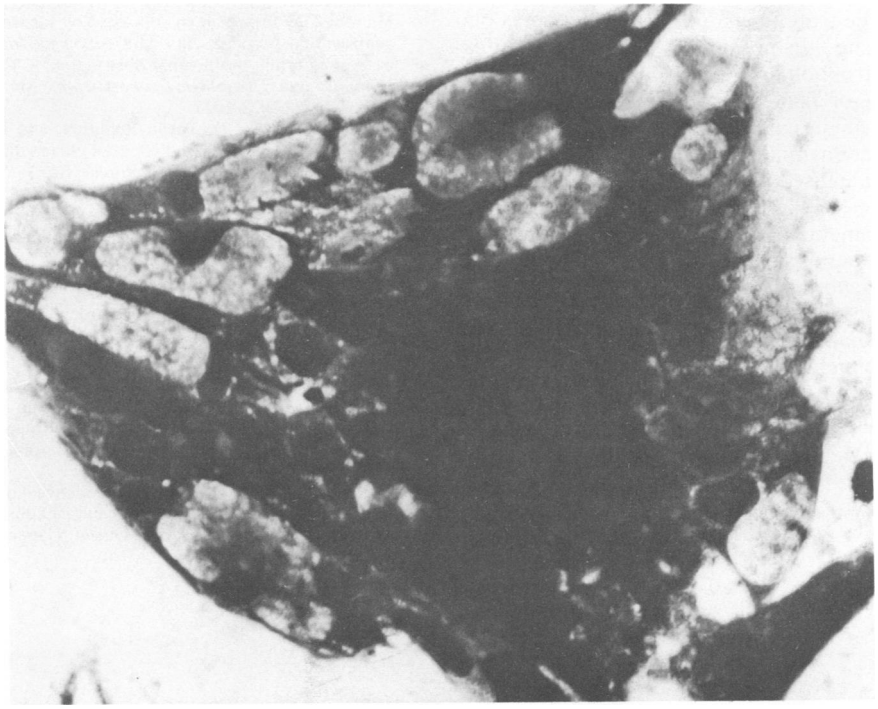

FIG. 2. Giemsa stain of mouse ova 48 h after infection with L2 serotype. Chlamydial inclusions appear as vacuoles containing granular material (the elementary bodies) in most trophoblast cells.

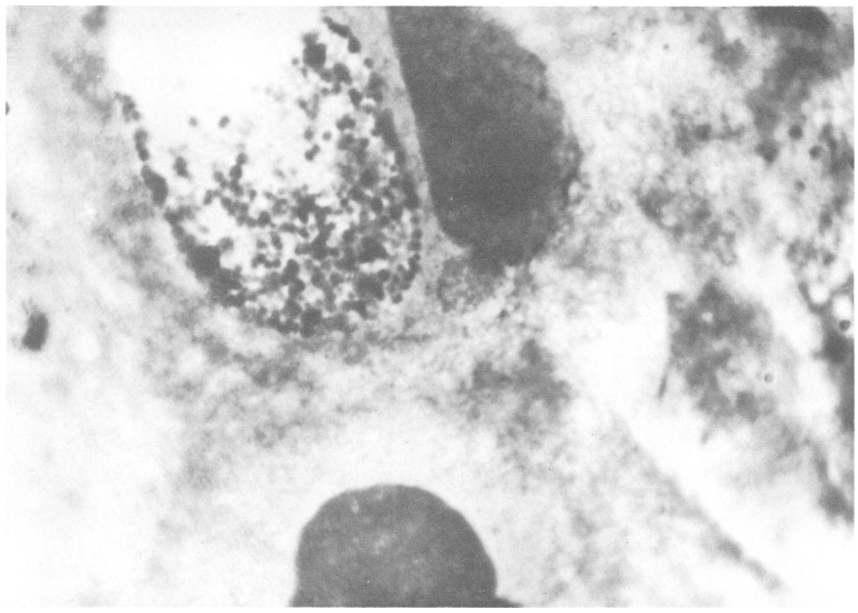

FIG. 3. Giemsa stain of same mouse ova as those shown in Fig. 2, with higher magnification showing elementary bodies with inclusion.

system and extrapolations to human in vivo infections must be made with great caution, it is of interest that the trophoblast cells, probably because they are phagocytic, were so readily infected in the absence of mechanical assistance. Since trophoblast ultimately becomes placental tissue, it is possible that chlamydial infection of trophoblast could interfere with the efficiency of placental attachment or function and thus might cause fetal damage or wastage.

In lower mammals, *Chlamydia psittaci* is an important cause of abortions (7). The mechanism varies with the host species, but placentitis is a prominent feature of some abortions, and uterine infections also occur (1, 7). A similar role for *C. trachomatis* in humans seems worth investigation. Earlier studies reporting recovery of *C. trachomatis* from human spontaneous abortion specimens were flawed because cervical contamination of the specimen was possible (4). These studies should be repeated with current technology.

This work was supported in part by the U.S. Department of Energy and by Public Health Service grants HD-12271 and EY-01205 from the National Institutes of Health.

LITERATURE CITED

1. **Bowen, R. A., P. Spears, J. Storz, and G. E. Seidel, Jr.** 1978. Mechanisms of infertility in genital tract infections due to *Chlamydia psittaci* transmitted through contaminated semen. J. Infect. Dis. **138**:95–98.
2. **Henri-Suchet, J., F. Catalan, V. Loffredo, et al.** 1980. Microbiology of specimens obtained by laparoscopy from controls and from patients with pelvic inflammatory disease or infertility with tubal obstruction: *Chlamydia trachomatis* and *Ureaplasma urealyticum*. Am. J. Obstet. Gynecol. **138**:1022–1025.
3. **Hutchinson, G. R., D. Taylor-Robinson, and R. R. Dourmashkin.** 1979. Growth and effect of chlamydiae in human and bovine oviduct organ cultures. Br. J. Vener. Dis. **55**:194.
4. **Schachter, J.** 1967. Isolation of bedsoniae from human arthritis and abortion tissues. Am. J. Ophthalmol. **63**:1082–1086.
5. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298**:428–435, 490–495, 540–549.
6. **Spindle, A.** 1980. An improved culture medium for mouse blastocysts. In Vitro **16**:669–674.
7. **Storz, J.** 1971. Chlamydia and Chlamydia-induced diseases. Charles C Thomas, Publishers, Springfield, Ill.
8. **Svensson, L., L. Westrom, and P.-A. Mardh.** 1981. Acute salpingitis with *Chlamydia trachomatis* isolated from the fallopian tubes: clinical, cultural and serologic findings. Sex. Transm. Dis. **8**:51.
9. **Westrom, L.** 1980. Incidence, prevalence and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am. J. Obstet. Gynecol. **138**:880–892.