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# *Mycoplasma pulmonis* Genital Disease: Effect of Rat Strain on Pregnancy Outcome

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***Background and Purpose:*** ***Mycoplasma pulmonis*** **is a natural pathogen of the respiratory and genital tracts of rats. Differential susceptibility and severity of the respiratory form of the disease, known as murine respiratory mycoplasmosis (MRM), exist between rat strains. We now report that specific rat strains vary in susceptibility to genital tract infection and pregnancy outcome.**

***Methods:*** **Specific-pathogen-free (SPF) female F344, LEW, Wistar (WIS) and Sprague Dawley (SD) rats were intravaginally inoculated with 3 x $10^7$ colony-forming units (CFU) of** ***M. pulmonis*** **strain X1048 or sterile diluent, and allowed to breed at 10 days after inoculation. Pregnant dams and pups were necropsied within 24 hours of parturition. At necropsy, culture for** ***M. pulmonis*** **was performed on dam and pups, and adverse effects on pregnancy outcome were assessed by determination of the incidence of infertility, fetal resorption, stillbirths, changes in litter size, and pup birth weight. Blood from dams was collected prior to inoculation and at time of necropsy for measurement of IgM and IgG antibodies to** ***M. pulmonis*****.**

***Results:*** **At time of necropsy, WIS (50%) and SD (60%) dams had a higher frequency of** ***M. pulmonis*** **culture positivity in the genital tract than did LEW (22.2%) and F344 (17.6%) dams. Dams that were still infected with** ***M. pulmonis*** **at time of necropsy had various complications. The SD rats had the greatest degree of adverse effects on pregnancy outcome, which were: infertility, decreased litter size ($P \leq 0.01$), decreased pup birth weight ($P \leq 0.01$), increased frequency of resorptions, stillbirths ($P \leq 0.05$), and the highest rate of pup pulmonary infection (23.1%) ($P \leq 0.001$). Despite a 50% colonization rate, WIS dams were the least adversely affected. The WIS pups born from** ***M. pulmonis*****-infected dams had slight decrease in birth weight, and only 6% had pulmonary infections. The LEW infected dams developed infertility and lower numbers of liveborn pups without evidence of vertical transmission. The F344 infected dams had lower numbers of liveborn pups that were smaller than their control counterparts, and none had pulmonary infections. None of the animals had detectable IgM and IgG antibodies to** ***M. pulmonis*** **before inoculation. At time of necropsy, all animals inoculated with** ***M. pulmonis*** **developed significantly ($P \leq 0.001$) higher amounts of** ***M. pulmonis*** **IgG and IgM antibodies, with SD rats developing the highest amounts ($P \leq 0.005$).**

***Conclusions:*** **Both F344 and LEW rats are more resistant to vaginal inoculation with** ***M. pulmonis*** **than are WIS and SD rats. However, only SD dams suffered severe adverse effects on pregnancy outcome. The SD dams also had the greatest IgM and IgG antibody response to** ***M. pulmonis*****. Our studies clearly indicate differences among rat strains in their susceptibility to vaginal inoculation with** ***M. pulmonis*** **and in secondary complications associated with infection. This system may be a useful model for determining host-specific factors that influence the outcome of natural mycoplasmal infections of the genital tract.**

Murine respiratory mycoplasmosis (MRM) caused by *Mycoplasma pulmonis* infection is a naturally acquired chronic disease of laboratory rats. Its severity is influenced by *M. pulmonis* strain, host environment, and host susceptibility to infection. Among rat strains, host susceptibility is variable in that Lewis (LEW) rats are more susceptible to MRM than are F344 rats (1–3). This difference has been exploited in studies focused on elucidating the host-specific factors that contribute to the pathogenesis of respiratory tract disease. Those studies have documented that LEW rats have higher non-specific lymphocyte responses and weaker *M. pulmonis*-specific immune responses than do F344 rats (1–3), which may account for the greater susceptibility and severity of MRM in LEW rats.

In addition to respiratory tract infections in rats, genital tract infections caused by *M. pulmonis* also have been reported (4–6). The LEW rats develop severe genital disease characterized by purulent endometritis, salpingitis, and perioophoritis (6). Conversely, conventionally housed Wistar rats from four separate breeding colonies were found to have naturally acquired subclinical genital mycoplasmosis; only 5% of infected animals had salpingitis and mild endometritis (5). In contrast, 40 to 50% of Sprague Dawley (SD) rats intravaginally inoculated with *M. pulmonis* develop genital tract disease (7–9). Pregnant SD animals develop placentitis and chorioamnionitis, and suffer adverse pregnancy outcome ranging from lower birth weight, smaller litter size, stillbirths, and fetal resorption (7–9). Although the wide range of disease severity described in those reports may be due to variable pathogenicity of *M. pulmonis* strains, different susceptibilities to genital mycoplasmosis among rat strains must also be considered.

We now report that LEW, F344, Wistar, and SD rats have distinct inherent susceptibilities to genital tract infection caused by vaginal inoculation with *M. pulmonis* strain X-1048. We found that LEW and F344 rats were more resistant to chronic colonization of the genital tract than were WIS and SD rats. However,

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once infection with *M. pulmonis* was established, all rat strains developed various complications, ranging from infertility, low pup weight, fetal resorption, and stillbirths. Of all rat strains studied, infected SD dams had the highest number of adverse outcomes, including resorption, stillbirths, in utero transmission of *M. pulmonis* to the fetus, and significantly lower live pup weight.

## Materials and Methods

**Mycoplasmas:** *Mycoplasma pulmonis* UAB strain X1048 was originally obtained from Maureen K. Davidson, University of Florida. To ensure identical inocula for all experiments, a large-volume culture was grown to late logarithmic phase in Frey's medium, aliquoted, and frozen at –80°C. Inocula were prepared by diluting thawed mycoplasmas in sterile phosphate-buffered saline (PBS) to obtain a concentration of 3 x $10^7$ colony-forming units (CFU)/100µl of medium. For each experiment, the number of CFU in each inoculum was verified by culture.

Unless otherwise noted, all cultures obtained from animals at necropsy were serially diluted 10-fold in Frey's broth to $10^{-5}$. For CFU determination, 20 µl from each sample and its corresponding dilutions (up to $10^{-2}$) were plated on Frey's agar. Agar plates were incubated at 37°C in 5% $CO_2$, and broth cultures were incubated at 37°C in ambient air. Broth tubes were examined daily for color change, and the reciprocal of the last dilution to show growth was deemed the color-changing unit (CCU). Agar cultures were incubated for at least five days before colonies were counted. All cultures were kept for 21 days before being discarded.

**Nested PCR for detection of *M. pulmonis* DNA:** Culture-negative uterine lavage specimens from inoculated dams were re-examined for the presence of *M. pulmonis* by use of polymerase chain reaction (PCR) analysis. The16S ribosomal sequence of *M. pulmonis* was detected by use of nested PCR analysis as described (10). For DNA extraction, uterine lavage specimens were centrifuged at 15,000 ×*g* for 15 minutes; the pellet was resuspended in lysis buffer (10 m*M* Tris, 100 m*M* KCl, 2.5 m*M* $MgCl_2$, 1% Tween 20, 1% Triton X-100, 1 mg of proteinase K/ml ) and incubated at 56°C for 4 hours. Proteinase K was inactivated by incubating the sample at 95°C for 15 minutes. Samples were centrifuged at 15,000 ×*g* for 15 minutes, and supernatant was collected and stored at -20°C until used for PCR analysis.

**Enzyme-linked immunosorbent assay for IgM and IgG antibodies to *M. pulmonis*:** Specific antibody to *M. pulmonis* was determined by use of an enzyme-linked immunosorbent assay (ELISA) as described (4). Briefly, all sera were diluted 1:100 in PBS containing 0.05% Tween 20 (PBSTA) and were tested in duplicate. For standardization, each microtitration plate included duplicates of positive- and negative-control rat sera. Serum (0.2 ml/well) was applied to 96-well microtitration plates coated with of *M. pulmonis* antigen (20 µg/ml/well), and incubated for 4 hours at 37°C. For detection, horseradish peroxidase-conjugated sheep anti-rat IgM and IgG (Southern Biotechnology, Birmingham, AL) were diluted 1:6,000 and 1:8,000, respectively, in PBSTA. The IgG subclasses were further identified, using sheep anti-rat $IgG_1$(diluted 1:1,000), $IgG_{2a}$ (diluted 1:2,000), $IgG_{2b}$ (diluted 1:1,000), and $IgG_{2c}$ (diluted 1:500) (Binding Site, Ltd., Birmingham, England). All detection antibodies were incubated overnight at room temperature. To develop the plates, 100 µl of a commercially available ABTS substrate solution (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was applied and incubated for 20 minutes at room temperature. To make comparisons between plates, the reaction was stopped with 50 µl of 1% sodium dodecyl sulfate when the absorbance readings for positive-control sera were equivalent. The ELISA plates were read by use of an automated microplate reader (model 3550, Biorad, Melville, NY) at a setting of 405 nm.

**Animals:** Specific-pathogen-free (SPF) male and female LEW, F344, SD, and WIS rats were purchased from a commercial vendor (Harlan Sprague Dawley, Inc., Indianapolis, IN). Rats were monitored and maintained free of the following pathogens: Sendai virus, H-1 virus, rat coronavirus, sialodacroadenitis virus, reovirus type 3, Kilham rat virus, Hantaan virus, *M. pulmonis*, respiratory tract and enteric bacterial pathogens, endoparasites, and ectoparasites. All animals were handled in accordance with procedures approved by the University of Florida Institutional Animal Care and Use Committee.

**Husbandry:** To maintain barrier conditions, all animals were handled within a laminar flow hood. Rats were housed in Microisolator® (Lab Products, Inc., Maywood, NJ) cages. Control animals were always handled first and housed separately from inoculated animals. All food, water, and caging was autoclaved before use.

**Processing and experimentally induced infection of rats:** Two rat strains were tested at the same time; outbred stocks (SD and WIS) and inbred strains (F344 and LEW) were examined simultaneously. All experiments in outbred stocks were repeated twice, and were repeated three times in inbred strains. For each experiment, a minimum of five females from each strain was used for each treatment group. All control and inoculated rats from the same strain were processed on the same day.

Female rats were anesthetized with a ketamine (Ketaset, Bristol Laboratories, Syracuse, NY) xylazine (Rompun, Haver-Lockhart, Shawnee, Kans.) cocktail (100 mg of ketamine and 150 mg of xylazine) administered intraperitoneally at a dosage of 0.1 ml/100 g of body weight. Prior to inoculation with *M. pulmonis*, blood was collected for *M. pulmonis* serologic testing. While anesthetized, animals were intravaginally inoculated with 100 µl of sterile Frey's broth or *M. pulmonis* UAB strain X1048 containing 3 x $10^7$ CFU in an equal volume of Frey's medium. To facilitate establishment of vaginal infection, animals were positioned so the pelvis was elevated for at least 20 minutes after inoculation.

Male SPF rats were processed at least three days prior to their introduction to females. Processing included methoxyflurane inhalant-induced anesthesia to facilitate ear notching and blood collection. Males were housed with other males when not used for breeding. A male rat was considered infected after exposure to an *M. pulmonis*-inoculated female. Therefore, *M. pulmonis*-exposed males were not exposed to control female rats, nor were they housed with any non-exposed males.

**Breeding and pregnancy detection:** At 10 days after inoculation, females were introduced to males of the same strain, at a cage density of two females per male. Females were examined daily for evidence of breeding by detection of presence of plug and spermatozoa in vaginal flush specimens. Bred females were separated from males within 24 hours of breeding. Pregnancy was confirmed by palpation at day 14 of gestation. Nonpregnant females were allowed to breed again before being considered infertile. The day after breeding was counted as day 1 of gestation. To prepare for parturition, females were housed singly by day 18 of gestation.

**Necropsy:** All dams and pups were necropsied within 24

hours of parturition. At that time, the total number of dead and live pups was recorded. Dead pups were considered stillbirths, and none were necropsied or cultured for *M. pulmonis*. Dams were deeply anesthetized by intraperitoneal administration of sodium pentobarbital (30 to 40 mg/kg of body weight), and were exsanguinated by transection of a femoral artery and vein. Specimens of trachea, vagina, and uterus from the dam were cultured for *M. pulmonis* as described (8).

The genital tract was examined for gross lesions, and the total number of implantation sites and retained fetuses were recorded. Numbers of implantation sites exceeding the number of pups were counted as resorptions.

All pups were euthanized by exposure to hypothermia. All, or a minimum of five liveborn pups from each litter, were randomly selected for necropsy. Oropharyngeal swab specimens for *M. pulmonis* culture were taken before necropsy. The skin was disinfected with 70% ethanol, and lung tissue was aseptically harvested for *M. pulmonis* culture.

**Statistical analysis:** Wherever possible, data were analyzed by use of analysis of variance. Fisher's multiple comparison test was used when results of analysis of variance indicated significant difference among group means. Contingency table analysis was used for comparisons between rat strains involving nominal data (positive vs. negative). For all analyses, a probability ($P$) $\leq 0.05$ was considered significant.

## Results

**Colonization of the dam by *M. pulmonis*:** *Mycoplasma pulmonis* was never isolated from any control rat at any time. For all animals, *M. pulmonis* PCR results matched culture results (data not shown). Table 1 summarizes the logarithmic number of CCU of *M. pulmonis* and its frequency of isolation from the genital and respiratory tracts of *M. pulmonis*-inoculated dams. Data analysis was restricted to CCU because several samples had excessively high CFU numbers that could not be accurately counted. Regardless of rat strain, all rats with culture-positive uterus also had culture-positive vagina. One LEW and one SD rat developed *M. pulmonis*-associated perioophoritis, and both were infertile. The SD dams had significantly ($P \leq 0.05$) higher CCU in the uterus than did F344, LEW, and WIS dams. The number of CCU in either tracheal or vaginal specimens was not significantly different among rat strains. Although results were not statistically significant, *M. pulmonis* was isolated from the genital tract of SD and WIS rats more often than from LEW and F344 rats. After combining the culture data from the first two experiments, only 2 of 10 F344, and 2 of 10 LEW rats were uterine culture positive at time of necropsy. From the third set of experiments, only 1 of 7 F344 and 3 of 8 LEW dams were culture positive for *M. pulmonis* at the time of necropsy. In this experiment, the F344 dam was culture positive for the trachea only, and two of the LEW dams were culture positive for the uterus and vagina.

**Pregnancy outcome:** The adverse effect of genital mycoplasmosis on each rat strain was assessed by differences in the number of liveborn pups, pup weight, and the frequency of adverse events (stillborn and resorptions) between control and infected rats. Since not all *M. pulmonis*-inoculated dams were culture positive for the genital tract at time of necropsy, we were concerned that this would not accurately document the affect of *M. pulmonis* on pregnancy outcome. To address this problem, we divided the data from *M. pulmonis*-infected dams into two groups: genital tract (vagina and uterus) culture-positive and genital tract culture-negative dams. For statistical analysis, pup data from control dams were separately compared with pup data from each subdivision of infected dams.

**Table 1.** Number of color changing units (CCU) of *Mycoplasma pulmonis* isolated from the respiratory and genital tracts of rats intravaginally inoculated with $10^7$ colony-forming units (CFU) of *M. pulmonis*[a]

| Rat Strain (n) | CCU Trachea (% positive) | CCU Vagina (% positive) | CCU Uterus (% positive) |
|---|---|---|---|
| F344 (17)[b] | 1.06 ± 1.95[d] (29.4)[f] | 0.88 ± 1.96[d] (17.6)[f] | 0.63 ± 1.71[d] (12.5)[f] |
| LEW (18)[b] | 0.39 ± 1.24[d] (22.2)[f] | 0.47 ± 1.38[d] (22.2)[f] | 0.61 ± 1.61[d] (22.2)[f] |
| SD (10)[c] | 0.7 ± 1.57[d] (30)[f] | 2.60 ± 2.55[d] (60)[f] | 2.70 ± 2.5[e] (60)[f] |
| WIS (10)[c] | 1.0 ± 1.76[d] (40)[f] | 1.50 ± 2.22[d] (50)[f] | 1.50 ± 2.41[d] (30)[f] |

[a]Values represent mean ± SD logarithm of the CCU of *M. pulmonis*. Cultures were obtained at time of necropsy, which occurred approximately 39 to 68 days after inoculation.
[b,c]Data are a combination of 3 (b) or 2 (c) separate experiments.
[d-f]Groups within the same column that share superscripts are not significantly different at $P \leq 0.05$.
Rat strains: F344 = Fischer 344; LEW = Lewis; SD = Sprague-Dawley; and WIS = Wistar.

The number of liveborn pups from control and *M. pulmonis*-inoculated dams is summarized in Table 2. Regardless of genital tract culture status, there were no differences between number of pups born to control and infected WIS dams. The number of liveborn pups born to genital tract culture-positive dams was reduced in F344, LEW, and SD rats. Because the small number of genital tract culture-positive dams in LEW and F344 groups prevented statistical analysis, we can only state that genital tract culture-positive SD dams had significantly ($P \leq 0.01$) lower numbers of liveborn pups. However, the large difference in litter size between LEW genital tract culture-positive dams and control dams strongly suggests *M. pulmonis* adversely affects this parameter in LEW rats.

Pup weight for the control group was compared with that for each subdivided category of pups born from uterine culture-positive and -negative dams (Table 3). Under these circumstances all F344, WIS, and SD pups born to *M. pulmonis* genital tract culture-positive dams were significantly ($P \leq 0.01$) smaller than their control counterparts. Of the three rat strains, SD pups born to genital tract culture-positive dams were the most severely affected ($P \leq 0.01$). Only three LEW dams were genital tract culture positive for *M. pulmonis*, and their pups weighed more than did pups born from LEW control rats.

Figure 1 summarizes the total number of adverse events (resorptions and stillbirths) in control and *M. pulmonis*-inoculated dams. Although it is not statistically significant, *M. pulmonis*-inoculated F344 dams had a slightly higher number of resorptions than did their control counterparts. Overall, *M. pulmonis*-inoculated SD rats had a significantly higher number of adverse events than did their control counterparts ($P \leq 0.05$); these included resorptions and stillbirths. There were no significant differences in the number of adverse events between control and *M. pulmonis*-inoculated LEW and WIS dams. Stillbirths were seen only in SD and WIS rats.

**Transmission of *M. pulmonis*:** *Mycoplasma pulmonis* was not isolated from any pups born to control dams. No LEW or SD pup had culture-positive oropharynx (data not shown). Six WIS pups born from genital tract positive dams had positive oropharyngeal culture results; 2 of these pups also had positive results of lung culture. Two F344 pups born to a genital tract culture-negative dam had positive oropharyngeal culture results; these pups had negative lung culture results.

No LEW pups had positive lung culture results; 2 of 73 F344, 2 of 32 WIS, and 6 of 26 SD pups had positive lung culture results. The SD dams had the greatest number of pups born with positive lung culture results ($P \leq 0.001$). The SD pups also had the greatest number of *M. pulmonis* organisms isolated from the lungs (data not shown) ($P \leq 0.001$).

**Antibodies to *M. pulmonis*:** The amount of anti-*M. pulmonis* IgG and IgM in the pre-infection sera of all rat strains was equivalent (data not shown). There were no differences between the amounts of anti-*M. pulmonis* IgG or IgM in the control rat sera obtained before inoculation and at necropsy (data not shown). Figure 2 summarizes the change in amounts of anti-*M. pulmonis* IgM and IgG in the sera of *M. pulmonis*-inoculated dams. At time of necropsy, the sera obtained from all *M. pulmonis*-inoculated animals had significantly ($P \leq 0.001$) higher amounts of anti-*M. pulmonis* IgG and IgM than before infection. Compared with other rat strains, SD rats developed the highest amounts of anti-*M. pulmonis* IgG ($P \leq 0.002$). Compared with other rat strains, SD rats had significantly ($P \leq 0.005$) higher amounts of anti-*M. pulmonis* IgG subclasses (data not shown).

## Discussion

We have reported that F344, LEW, WIS, and SD rats significantly differ in their susceptibility to genital infection with *M. pulmonis* strain X1048. Establishment of a chronic genital tract infection in F344 and LEW rats by vaginal inoculation was extremely difficult despite repeated efforts, suggesting that these two rat strains are resistant to the genital disease under the experimental infection conditions used in our study. This is a particularly intriguing observation given the documented susceptibility of LEW rats to MRM. Although SD and WIS rats were more susceptible to colonization with *M. pulmonis*, only SD rats developed severe pregnancy complications associated with genital mycoplasmosis, which included increases in the numbers of adverse effects on pregnancy outcome and a greater frequency of in utero transmission of the organism to the fetus. These significant differences between rat strains are not due to variation in inoculating dose because all animals received the same number of organisms from the same stock culture of *M. pulmonis*.

Vaginal inoculation of *M. pulmonis* in F344 and LEW rats resulted in self-limiting genital tract infections. Like mice, these rat strains may be useful for studying the effect of reproductive hormones on innate immunity of the genital tract. In mice, reproductive hormones and stage of estrous cycle influence susceptibility to genital mycoplasmosis (11–13). Estrogen facilitates clearance of *M. pulmonis* colonization from the genital tract or prevents infection, whereas progesterone treatment enhances establishment of genital infection in mice (11, 12). Mice intravaginally inoculated with *M. pulmonis* during diestrus are more susceptible to infection than during estrus (12). Since reproductive hormone concentrations and vaginal epithelium change during estrous stage, it is possible that F344 and LEW rats have variable susceptibility to genital mycoplasmosis at different stages of the estrous cycle. Further work would be required to determine whether this could significantly affect colonization of the genital tract in both LEW and F344 rats.

The LEW rats are acknowledged as the strain most susceptible to mycoplasmal respiratory tract infection (1–3). However, in our study, they had unexpected resistance to establishment of chronic genital tract infection by vaginal inoculation. Although LEW rats were relatively resistant to vaginal infection, those rats in which genital infection was established developed infertility and had decreased litter size. Natural *M. pulmonis* infection in LEW rats is associated with infertility, metritis, salpingitis, and perioophoritis (6). Results of our current experimentally induced infection study coupled with the observations reported in natural infections (6) imply that *M. pulmonis* genital tract disease in LEW rats is mostly associated with ovarian disease and, possibly, prevention of fetal implantation. In natural infections (6) as well as our present experimental infection study, LEW rats with genital tract infection also had MRM as well as multiple infected sites. Therefore, naturally acquired genital myco-

**Table 2.** Number of liveborn pups per control and *M. pulmonis*-inoculated dams[a]

| Strain | Control(n) | Inoculated (n) | Genital tract colonization of inoculated dams[b] | |
|---|---|---|---|---|
| | | | Positive (n) | Negative (n) |
| F344 | $7.9 \pm 3.3^{c}$ (7) | $8.1 \pm 2.5^{c}$ (17) | $5.3 \pm 2.1^{e}$ (3) | $8.3 \pm 2.7^{c}$ (14) |
| LEW | $9.4 \pm 3.5^{c}$ (7) | $8.2 \pm 4.2^{c}$ (18) | $1.3 \pm 1.2^{e}$ (4) | $9.7 \pm 2.8^{c}$ (14) |
| WIS | $12.6 \pm 4.7^{c}$ (5) | $12.6 \pm 4.2^{c}$ (10) | $13.2 \pm 2.5^{c}$ (5) | $12.7 \pm 5.0^{c}$ (5) |
| SD | $11.6 \pm 2.8^{c}$ (7) | $5.6 \pm 7.4^{c}$ (10) | $2.7 \pm 6.5^{d}$ (6) | $11.2 \pm 6.7^{e}$ (4) |

[a] Values are the (mean ± SD) number of live pups born from control and *M. pulmonis*-inoculated dams. Numbers in parenthesis represent the number of dams in each group. Data from WIS and SD groups are a combination of two separate experiments. Data from F344 and LEW groups are a combination of three separate experiments.
[b] Data from *M. pulmonis*-inoculated dams were subdivided into the number of liveborn pups born to genital tract culture-negative dams and genital tract culture-positive dams. Cultures from dams were obtained at time of necropsy, which occurred approximately 39 to 68 days after inoculation.
[c,d] Groups within the same row that share superscripts are not significantly different at $P \leq 0.01$.
[e] The small sample size precluded statistical analysis.
*See* Table 1 for key.

**Table 3.** Weight of pups (grams) born to control and *M. pulmonis*-inoculated dams[a]

| Strain | Control(n) | Inoculated[b] (n) | Genital tract-colonized inoculated dams[c] | |
|---|---|---|---|---|
| | | | Positive (n) | Negative (n) |
| F344 | $5.39 \pm 0.47^{d}$ (55) | $5.56 \pm 0.43^{e}$ (137) | $5.14 \pm 0.43^{f}$ (9) | $5.59 \pm 0.42^{e,g}$(128) |
| LEW | $5.67 \pm 0.76^{d}$ (69) | $5.79 \pm 0.76^{d}$ (141) | $6.96 \pm 1.20^{h}$ (4) | $5.78 \pm 0.75^{d}$ (137) |
| WIS | $6.34 \pm 0.84^{d}$ (63) | $6.20 \pm 0.50^{d}$ (130) | $5.98 \pm 0.57^{e}$ (41) | $6.30 \pm 0.43^{d}$ (89) |
| SD | $6.24 \pm 0.47^{d}$ (85) | $5.57 \pm 1.15^{e}$ (63) | $4.25 \pm 0.43^{f}$ (23) | $6.30 \pm 0.59^{d}$ (40) |

[a] Values are the (mean ± SD) live pup weight (grams) born to control and *M. pulmonis*-inoculated dams. Numbers in parenthesis represent the number of pups in each group. Pup weight was obtained within 24 hours of parturition. Data from F344 and LEW rats are a combination of 3 separate experiments. Data from WIS and SD rats are a combination of 2 separate experiments.
[b] Values include pup weight from all dams in the *M. pulmonis*-inoculated group, regardless of *M. pulmonis* genital tract culture status. Dams were inoculated with *M. pulmonis* approximately 39 to 68 days prior to pup birth.
[c] Pup weight data from *M. pulmonis*-inoculated dams were subdivided into *M. pulmonis* genital tract culture-positive and *M. pulmonis* genital tract culture-negative groups.
[d-g] Groups within the same row that share superscripts are not significantly different at $P \leq 0.01$.
[h] The small sample size precluded statistical analysis.
*See* Table 1 for key.

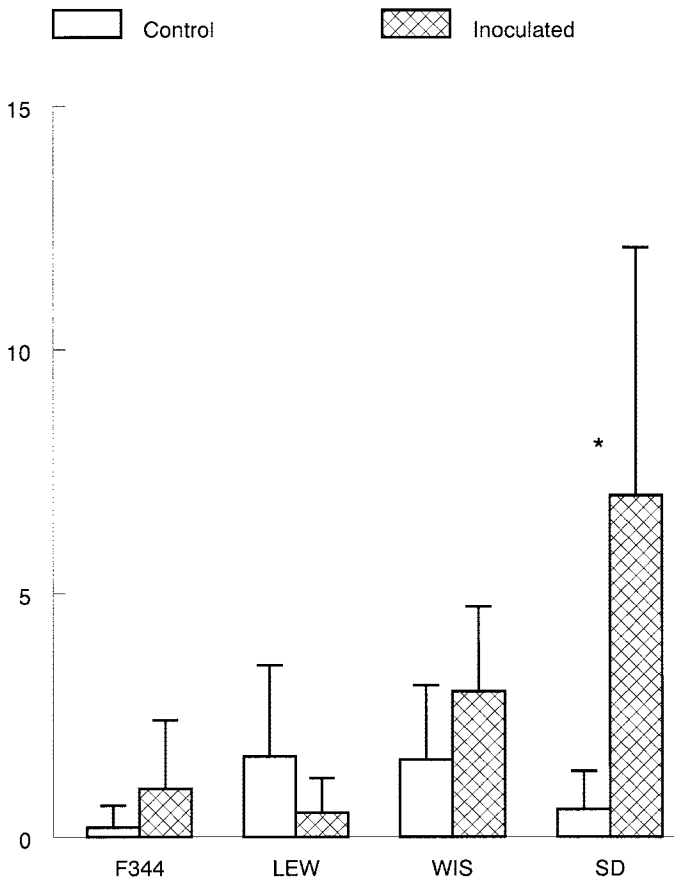


**Figure 1.** Mean ± SD total number of adverse events (resorptions and stillborn) in control and *Mycoplasma pulmonis*-inoculated dams. Data from Sprague-Dawley (SD) and Wistar (WIS) rats are a combination of two separate experiments. Data from Fischer 344 (F344) and Lewis (LEW) rats are a combination of three separate experiments. *The number of adverse events between control and *M. pulmonis*-infected SD dams was significantly ($P \leq 0.001$) different.

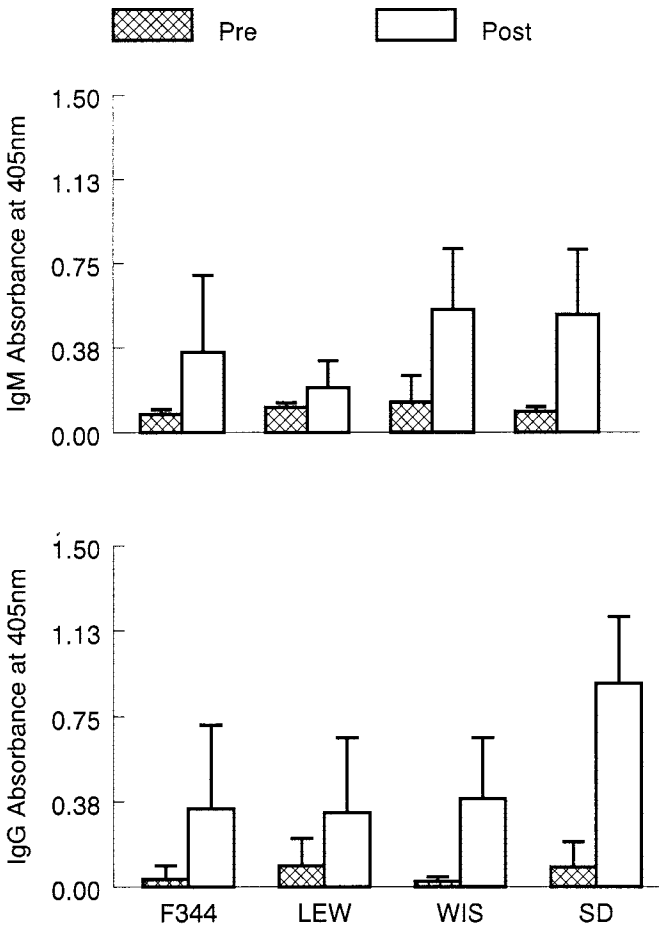


**Figure 2.** *Mycoplasma pulmonis*-specific IgM and IgG from the sera of pre-and postinoculated rats. Antibody concentrations are expressed as mean ± SD absorbance value obtained from ELISA. Each group contained samples from at least five animals. After inoculation, all rat strains had significantly ($P \leq 0.001$) higher amounts pf *M. pulmonis*-specific IgM and IgG. Compared with other rat strains, SD rats produced the highest amounts of *M. pulmonis*-specific IgG ($P \leq 0.002$).

plasmosis in LEW rats may be a sequella to systemic infection as opposed to ascending infection of the genital tract. Further studies involving intravenous inoculation of *M. pulmonis* before and during various stages of pregnancy would be necessary to test this hypothesis.

Others have reported that 30% of WIS dams obtained from 4 conventional breeding colonies had subclinical *M. pulmonis* genital tract infections (5). Our results parallel their findings in that we were able to experimentally establish an infection rate of 30 to 50% in WIS dams. The only adverse effect we noted in our study was slight decrease in WIS pup birth weight. Although WIS dams had similar rates of genital tract infection as did SD dams, WIS dams were not as severely affected. This may be due to the fewer number of *M. pulmonis* organisms isolated from the genital tract of WIS dams or the ability of these animals to contain *M. pulmonis* infection to the mucosal surface of the genital tract. The low incidence of adverse events and low rate of pup infection in WIS rats suggest that neither mycoplasmaemia nor breach of the placental barrier occurred in these animals. Mycoplasmaemia may be necessary for in utero infection of the fetus since fetal infections can be readily established in pregnant rodents that are intravenously inoculated with *M. pulmonis* (14). Furthermore, the few WIS pups born with positive lung culture results also had positive oropharyngeal culture results. These infections could have been acquired by aspiration of *M. pulmonis* during birth instead of via in utero transmission.

Of the rat strains studied, SD dams had the most marked decreases in birth weight, the highest rate of in utero transmission to the fetus, and the highest amounts of *M. pulmonis*-specific antibody. Previous studies have documented that SD dams intravaginally infected with *M. pulmonis* develop placentitis and chorioamnionitis, with subsequent in utero transmission of *M. pulmonis* to the fetus (7–9). It has been postulated that placental infection with *M. pulmonis* in SD dams is a result of ascending infection (7); however, mycoplasmemia with breach of the placental barrier must also be considered. In our study, SD dams had the highest amounts of *M. pulmonis*-specific antibody in serum; significant increases in antibody responses to infection correlate with bacteremia (15) and mycoplasmaemia (16). Moreover, previous studies with SD rats documented fetal loss from *M. pulmonis* to be highest in animals intravaginally inoculated with *M. pulmonis* before breeding, which established chronic infection, and at day 14 of gestation, when placental circulation is established (7). Further work is necessary to determine whether the adverse effects in our experiments were due to myco-

plasmemia as opposed to ascending infection of the genital tract, or a combination of both routes of exposure.

Most mycoplasmal infections are persistent and subclinical (16, 17). Overt disease is probably due to a combination of factors encompassing microbial pathogenicity, environment, and host immunity. In humans, mycoplasmal infections of the genital tract can be asymptomatic or manifest with several complications, such as infertility, low birth weight, prematurity, neonatal sepsis, and death (14, 16–18). Epidemiologic studies indicate a correlation between ureaplasmaemia, seroconversion, and symptomatic amnionitis (16, 17), but the mechanisms involved in development of ureaplasmaemia are still unknown. Although any animal model has limited usefulness, *M. pulmonis* is a naturally acquired infection of the genital tract in rats. The host-pathogen relationship between *M. pulmonis* in rats may be similar to that of *U. urealyticum* in humans. The rat strain differences we noted in our study, especially between WIS and SD dams, may be useful in elucidating the host-specific mechanisms that affect resistance and overt genital tract disease.

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