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Evaluation of the role of exogenous pathogens on the incidence of embryo loss during early pregnancy in mice

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Abstract

The mating of CBA/j♀ mice ($H2^k$) by DBA/2j♂ mice ($H2^d$) typically results in an elevated incidence of spontaneous embryo loss thus providing an ideal genetically controlled laboratory model for the study of the factors causing early embryo loss during pregnancy. There is now considerable data on the cells and factors involved in fetal resorption but little is known about the events which activate this process. While the activation of the maternal response to the fetal implant could have endogenous or genetic origins, a role for exogenous factors including microbial pathogens could also be involved. In order to investigate these possibilities, the reproductive success of CBA/j♀ × DBA/2j♂ matings in a conventional animal care facility were compared with matings in a specific pathogen free (SPF) animal facility. All animals housed under these conditions were routinely screened by immunoassay and culture, for the presence of a number of viral and bacterial pathogens of mice. The incidence of spontaneous embryo loss in specific pathogen free CBA female mice mated by DBA and other male strains was found to be virtually identical to that of CBA female mice infected with multiple viral pathogens and housed under otherwise identical conditions (non-SPF). However, the numbers of implantation per pregnancy was significantly greater in an SPF facility. Therefore, exposure of mating mice to exogenous viral and bacterial pathogens did not appear to alter the overall incidence of spontaneous embryo resorption. It was concluded that the immunomodulatory effects of infection by common murine pathogens neither augmented nor reduced post-implantation embryo losses.

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1. Introduction

The data on early embryo losses in domestic and experimental animals is generally consistent with that for human losses (reviewed in Baines and Gendron, 1990, 1993). Many domestic species show early embryo loss rates in the range of 25–40% which is also seen in early human pregnancy. Whereas human embryo loss usually results in the expulsion of the products of conception, in animal species which bear litters, the early embryos may be partly or completely resorbed during the gestational period. Only later fetal deaths result in stillbirths or actual abortion. The question remains as to the actual causes of early embryo loss. Several murine laboratory models exist for the study of the biology of spontaneous embryo loss. The mating of CBA/j ♀ ($H2^k$) by DBA/2j ♂ ($H2^d$) provides an ideal model in which 20–30% of embryonic implants are lost before mid-gestation (Chaouat et al., 1983; Kiger et al., 1985; De Fougerolles and Baines, 1987; Clark et al., 1987). Conversely, syngeneic CBA/j ♀ × CBA ♂ matings and matings of CBA/j ♀ by BALB/c ♂ ($H2^d$) show loss rates below 10%, as do many other inbred and outbred strains of mice housed under specific pathogen free (SPF) conditions. However, recent studies by Krackow (1992) have confirmed the early observations of Hollander and Strong (1950), that in most outbred and inbred strains of mice housed in conventional facilities, about 15% of the embryos resorb. The reason for this difference in normal resorption frequencies is not clear but could be due to either genetic factors related to the strains studied or exposure to the environmental pathogens commonly present in conventional animal care facilities. These data question our assumptions of what constitutes the incidence and causes of 'normal' embryo loss in laboratory mice. One might explain the normally low embryo loss rates in inbred and pedigreed strains of mice on the basis of either their genetic nature or their reduced exposure to environmental pathogens. Inbred mice are genetically selected for defect free genetic purity, high fertility, large litter size and low loss rates. In addition, these mice are line bred under scrupulous pathogen free conditions so that even subtle pathogens are not passed from one individual or strain to another. It is not clear what role genetic (endogenous) factors or environmental pathogens (exogenous factors) play in determining the low embryo loss rates currently observed in laboratory animal breeding programs.

Current estimates of the involvement of exogenous pathogens as causes of later spontaneous pregnancy losses in humans fall in the range of 3–10% of all conceptions (Benirschke and Robb, 1987). No comprehensive data exist

for early human embryo losses prior to 6 weeks. In studies of the CBA/j ♀ × DBA/2j ♂ model, the losses occur around day 10–12 of pregnancy, starting barely 5 days after implantation. The effector cells are not antigen specific thymus derived cytolytic lymphocytes as one may expect, but non-specific natural killer lymphocytes and macrophages (Gendron and Baines, 1988; Kinsky et al, 1990). Failing feto-placental implants lack an essential immunosuppressive activity that normally regulates decidual natural killer cell activation (Gendron et al., 1990). Furthermore, it is clear from animal studies that the early exposure of gravid females to bacterial products such as lipopolysaccharides (LPS) during the early post-implantation period can induce precipitous total pregnancy loss (Zahl and Bjerknes, 1943; Chedid et al., 1962; Lanning et al., 1983; Parant, 1990; Gendron et al., 1990b). Viral agents which can cross the placenta and infect the embryo, may also cause intrauterine death of the embryo though the actual expulsion of the placental sac may occur some time later. Viral infections and graft versus host responses may also reduce the effectiveness of gastrointestinal defenses, permitting increased gut permeability which would allow the penetration of bacterial LPS into the bloodstream (Lapp, personal communication). Significant increases in serum LPS during pregnancy would result in total embryo loss. It appears that total early embryo loss may be due to an acute failure of placental development even though the embryo may be normal (Surani et al., 1984, 1988). LPS and cytokine induced pregnancy losses appear to involve a similar acute damage to placental development and embryo loss (Croy and Summerlee, 1990; Gendron et al., 1990; Lanning and Hilbelink, 1984). While certain bacterial pathogens may cause the total loss of all implanted embryos, the CBA/j ♀ × DBA/2j ♂ model involves partial litter loss implying that the inducing agents and cellular mechanism may be different.

This study examined the possible effects of environmental factors on the incidence of embryo loss in laboratory mice. In order to study this question we examined the rates of early embryo losses in a specific pathogen free colony of inbred and outbred mice and compared the results with data from a chronically infected conventional colony. Our results show that the pathogen status or season of the year have little or no effect on the incidence of early spontaneous embryo loss.

2. Material and methods

2.1. Animals

Mice for these studies were obtained at 8–10 weeks of age and acclimatized to the animal facility for 2 weeks prior to use. CBA/j females and BALB/c males were obtained from Jackson Labs (Bar Harbor, Maine), DBA/2 males and CFW ♂ mice from either Jackson Labs or Charles River, (St. Constant,

Quebec). These animals were housed in the McGill Animal Care Facility in the Department of Microbiology and Immunology in accordance with the guidelines provided by the Canadian Council for Animal Care. The laboratory mice had unlimited access to food and water in a climate controlled environment with illumination from 07:00 h to 19:00 h. As described in detail below, this facility was not pathogen free prior to 20 April 1989. Following a successful decontamination of the facility at that time, the facility was restocked with pathogen free mice and has been free of all specific pathogens of mice and other experimental species since 1989.

2.2. Animal care facility before decontamination (pathogen infected)

The departmental animal care facility was a conventional research animal housing area consisting of several interconnecting rooms in which several species were simultaneously housed (mice, rats, hamsters, gerbils, chickens and rabbits). Different strains of mice from different suppliers were placed in rooms without any efforts to quarantine or isolate them from existing stocks. Staff and users were admitted to all areas without any specific disinfection procedures. Access doors at the front and rear of the facility permitted a large number of unauthorized staff to 'walk-through' the facility. Food and water was provided ad libitum to all animals regardless of their source or experimental status. No sentinel program for monitoring the presence of pathogens existed.

Serological and bacteriological tests for murine pathogens by the Institute Armand Frappier (Laval-des-Rapides, Quebec) and Charles River Professional Services indicated the presence of several pathogens in the animal facility including murine hepatitis virus (MHV), minute virus of mice (MVM), Theiler's virus (GDVII), occasionally mouse adenovirus and often *Mycoplasma* species. The rats were positive for Sendai virus (SV). Serological assays were generally performed by ELISA and confirmed by immunofluorescence tests on infected tissue culture cells.

2.3. Decontamination process

All unnecessary stocks of animals were utilized and/or eliminated in the months prior to the decontamination of the facility. The rooms and caging units were all thoroughly cleaned and then washed with a surface disinfectant solution and allowed to thoroughly dry for 1 week. All equipment and areas were examined for potential sources of pathogens and either eliminated, autoclaved or disinfected. A sentinel program was initiated and sera were monitored monthly for the first 6 months.

2.4. Animal facility after decontamination (specific pathogen free)

The facility has been isolated to limit entry to only authorized users and

staff. All new animal stocks are ordered from suppliers who supply a certificate of pathogen free status and ship them in filter protected containers. The filter protected containers are surface disinfected prior to removal of the mice and they are placed in conventional plastic boxes with food and water freely available. Animals received from different suppliers are placed in separate stock rooms for each supplier and are only dispensed by animal care personnel. All animal users don clean surgical gowns and booties on each entry and routinely disinfect their hands and work area before, during and after each procedure. A strict prohibition against the admission of potentially infected materials is enforced. Any animals removed from the facility may not be returned to the facility and no biologicals are admitted without a prior check of their pathogen free status (e.g. cell lines, transplantable tumors, parasites or hybridomas or their products). Experiments with microbial agents which are not normal murine pathogens are permitted in an isolated suite of negative airflow rooms which includes a self-contained autoclave. For experiments with any infectious agents the usual sterile practices are rigorously followed.

2.5. Serological and direct screening for exposure to pathogens

Sera from both sentinel mice and the experimental mice were initially submitted to two professional laboratories for comparative serological analysis. The results from Charles River Professional Services and Armand Frappier Diagnostics (Laval-des-Rapides, Quebec) were closely comparable and the latter now performs our routine serological screening. Initially monthly full spectrum screening for all murine pathogens were done followed by intermittent MHV testing. The annual full panel analysis tests for exposure to MHV, Ectromelia, Epidemic Diarrhoea Virus of infant mice, Theiler's Virus, K Virus, Lymphocytic Choriomeningitis Virus, Murine Adenovirus (MAd), Murine Cytomegalovirus, Mouse Thymic Virus, MVM, Pneumonia virus of Mice, Rheovirus-3, SV and *Mycoplasma pulmonis*. Sentinel bleeds are now performed quarterly. Additional bacteriological cultures are performed as required.

2.6. Assessment of fetal losses

Four female mice were housed with one male in a standard plastic mouse box and the females were checked daily for the presence of a vaginal mating plug. The day of sighting of the plug was arbitrarily designated as day 0. The majority of early embryo losses occur between day 9 and day 12. Generally, the mated females were killed by cervical dislocation and the uterus examined on day 12, though losses were also studied between day 6 and day 21. The total number of implantation sites were counted and those that were resorbing were separately recorded. A resorbing implantation site was usual-

ly significantly smaller than the majority of healthy implantation sites, with signs of anoxia, ischemia and hemorrhage, often with evidence of separation of the embryo from the uterine wall. The loss of embryonic structures and organization was confirmed by histology in some cases. No attempt was made to count implantation scars where early embryos may have been totally resorbed. Later losses were defined as those seen after day 14 and usually involved the intrauterine death of an intact fetal mouse pup. Such later fetal deaths are not resorbed by the uterine tissues but are stillborn.

2.7. Data analysis

The total number of implantated embryos per uterus (total of the viable and resorbing embryo sites) was recorded for each female and averages and standard deviates for each group were calculated. The percentage of embryos resorbing in each female was calculated and the averages and standard deviates for each group were computed. After establishing that the homogeneity of the variance for the groups existed using Bartletts test, individual groups were compared using one-way ANOVA and Tukey-Kramer multiple range tests.

3. Results

3.1. Spontaneous embryo resorption occurs by day 12 of gestation in infected mice

The interstrain mating of CBA females with DBA males in the conventional animal care facility prior to decontamination (non-SPF), resulted in

Table 1
Embryo losses for CBA/j females mated by syngeneic or allogeneic males

Female × male mating pair	No. mice	Embryos per uterus (day 12 of gestation)	% Embryos resorbed
CBA/j♀ × DBA/2j♂	39	7.8 ± 2.3	23.8 ± 15.4
CBA/j♀ × BALB/cj♂	12	5.6 ± 1.4	5.3 ± 7.8
CBA/j♀ × CBA/j♂	12	6.0 ± 0.6	5.2 ± 7.3
CBA/j♀ × CFW♂	12	9.3 ± 1.1	2.6 ± 6.9

Data are presented as the mean and standard deviation. All matings for this table were performed prior to the decontamination of the animal facility on mice exposed to the endemic pathogens described in the text. For CBA/j females mated by the respective males, the number of implantations and resorptions per uterus were assessed on day 12 following mating. No attempt was made to count implantation scars where early embryos may have been totally resorbed. Resorbing embryos on day 12 were typically small for date, hemorrhagic and eventually necrotic.

the implantation of 8–10 embryos per uterus and subsequently 20–30% of these embryos abruptly resorbed between days 10 and 13 (Table 1). This ultimately resulted in the delivery of an average of seven viable newborn pups per litter (data not shown). Only matings of CBA/j♀ by DBA/2j♂ showed an incidence of embryo loss of about 20% whereas, CBA/j♀ mated by BALB/c, CBA♂ or CFW♂ exhibited normal implantation rates and low frequencies of embryo loss.

An examination of the time course of early embryo loss in the CBA/j♀ mouse indicated that fetal resorption was an acute event starting at day 9 and terminating at day 11 of gestation with few fetal losses occurring between day 12 and the day of delivery (Table 2). While the distribution of the resorptions among the female mice was random, the mean incidence for embryo resorption for our facility was 21.33 ± 14.18% (S.D.) and the average number of implantations per uterus was 8.93 ± 2.08 (S.D.). Fig. 1 shows that the

Table 2
Embryo implantations and losses during murine gestation

Days post coitum	No. of CBA/j female mice	Embryos per uterus	% Embryos resorbed
8	10	9.8 ± 2.1	Not detectable
9	9	10.0 ± 1.6	5.4 ± 6.6
10	11	10.4 ± 1.7	15.3 ± 10.0
11	40	7.6 ± 2.4	22.9 ± 17.3
12	23	8.1 ± 2.6	23.3 ± 14.0
13	16	7.4 ± 2.5	24.6 ± 24.0
14	8	5.7 ± 0.8	8.5 ± 11.9
15	14	7.2 ± 2.1	7.5 ± 14.7
16	14	7.6 ± 2.5	14.9 ± 16.8
17	8	7.7 ± 2.1	4.3 ± 6.0
18	4	8.8 ± 0.8	2.2 ± 4.4
19	4	7.3 ± 2.5	10.3 ± 15.7

Data are presented as the mean and standard deviation. All mating for this table were performed prior to the decontamination of the animal facility on mice exposed to the pathogens described in the text. In CBA/j females mated by DBA/2j males, the number of implantations and resorptions per uterus were assessed on the days indicated. Since early embryo losses were not detectable from the time of implantation on day 5 to day 8, no attempt was made to quantify losses at these times. No attempt was made to count implantation scars where early embryos may have been totally resorbed. Resorbing embryos from day 9 to 13 were typically small for date, hemorrhagic and eventually necrotic. Resorptions counted after day 14 were primarily those embryos which have clearly died in utero subsequent to normal placental development. These later counts of embryo losses include only intrauterine death and stillbirths.

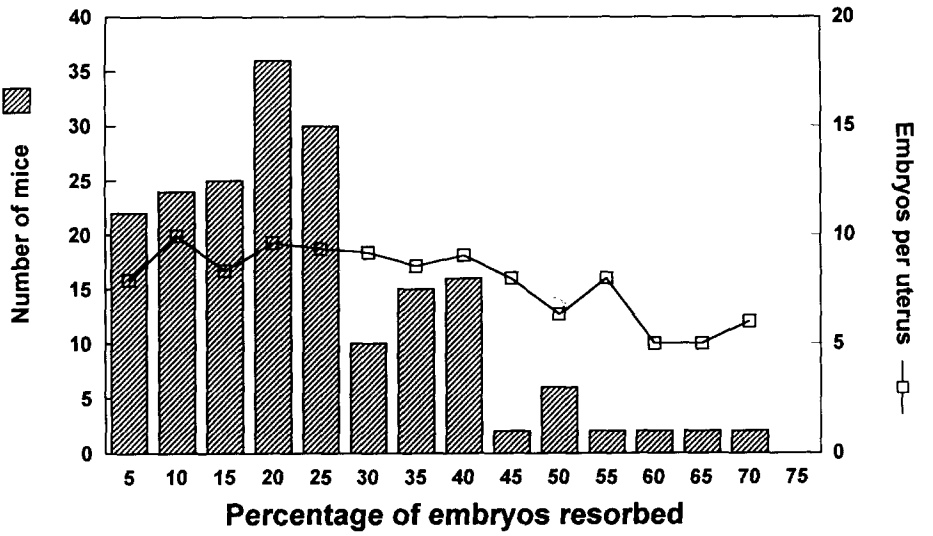

Fig. 1. Plot of the frequency distribution of the numbers of CBA/j ♀ mice mated by DBA/2♂, showing the percentages of embryo loss for each female at day 12 (increments of 5%). The matings presented in this figure were accumulated between 1985 and 1988 for the conventionally housed mice which were exposed to, and presumably infected by, multiple pathogens. The mean incidence of early embryo resorption for the entire group of CBA/j ♀ mice was 21.33 ± 14.18% (S.D.), with no females showing greater than 75% loss of a single litter (range 0–75% loss, 95% confidence limits 6–49% loss). The average number of embryos implantated is also plotted for each group and the overall mean was 8.93 ± 2.08 (S.D.) embryos per uterus.

occurrence of resorptions was distributed in a statistically normal fashion and that the numbers of implantations was similar for all females regardless of outcome. Those female mice with losses exceeding 55% of the embryos showed lower numbers of implants but the numbers of female mice in these groups were very low and the difference was not significant.

Analysis of other reproductive parameters in CBA/j ♀ by DBA/2j♂ matings, revealed no significant month to month variation in either implantation or resorption rates and no seasonal variability (Table 3). Thus, the CBA/j ♀ by DBA/2j♂ matings provided a unique model for the study of the factors causing early embryo losses which occurred during pregnancy.

3.2. Multiple pathogen exposure status of conventionally housed animal stocks

In order to determine whether the pathogen exposure status of the mice accommodated within this facility affected their reproductive performance, the implantation and resorption rates before and after the successful elimination of several endemic pathogens were compared. A full serological and

Table 3
Monthly incidence of embryo implantation and percent resorption

Month mice were mated	No. of CBA/j female mice	Embryos per uterus	% Embryos resorbed
January	24	8.3 ± 2.3	15.6 ± 9.0
February	8	10.1 ± 1.7	27.4 ± 9.9
March	7	9.0 ± 2.1	25.2 ± 17.0
April	9	9.0 ± 2.1	24.7 ± 8.7
May	23	8.6 ± 2.0	22.4 ± 11.9
June	28	8.1 ± 2.3	17.0 ± 12.7
July	59	9.1 ± 2.2	17.4 ± 14.0
August	27	8.7 ± 2.3	25.7 ± 17.7
September	14	9.4 ± 1.3	24.1 ± 10.3
October	7	9.4 ± 1.7	12.7 ± 10.0
November	13	8.6 ± 0.9	22.3 ± 17.2
December	16	8.9 ± 1.9	21.4 ± 12.6
Overall averages		8.9 ± 2.1	21.3 ± 14.2

Data are presented as the mean and standard deviation. See legend to Table 1 for general methodology.

histopathological screen of the health status of the animals within this facility before the clean-up was initiated. This identified the presence of several endemic diseases including MHV, MVM, MAd, mycoplasma species (MYCO) and SV. The rats in the same facility were found to be infected with Sialodacryoadenitis virus and GD-VII virus and the rabbits were infected with *Mycoplasma* and possibly *Pasteurella* and *Yersinia* species. Though unconfirmed by serology, the nature of the pathology in our in-house breeding programs indicated that there could in fact have been several concomitant types of MHV present including an avirulent MHV (type 1 or 2) and a recently introduced highly virulent MHV (possibly type 3). The pathogen, which caused massive neonatal mortality, was introduced to this facility with some transgenic mice which had been serologically screened by another laboratory and were incorrectly stated to be pathogen free. However, the mice were negative for ectromelia virus, K virus, lymphocytic choriomeningitis virus, murine cytomegalovirus, mouse thymic virus, mouse polyomavirus, pneumonia virus of mice, reovirus type 3 and epidemic diarrhea virus of infant mice. Bacteriological cultures of the urine, feces and tissues from the mice for common pathogens showed no unusual results. Subsequently, all pathogens were successfully eliminated in April 1989 and the animal care

facility has been maintained as a specific pathogen free facility for over 4 years.

3.3. Pregnancy losses were unaltered by elimination of exogenous pathogens

Prior to the disinfection of the animal care facility, CBA female mice mated to DBA/2 male mice showed the expected high rate of early embryo losses as compared with matings of CBA/j♀ × CBA♂, CBA/j♀ × BALB/c♂ and outbred CFW♀ × DBA/2♂. However, the implantation rate in 1985 was significantly lower than the number of embryos per uterus observed after the decontamination of the facility (Table 4). The resorption rates for all years studied, while somewhat erratic due to the relatively small sizes of some of the samples, were well within the ranges expected. The successful pathogen eradication procedure took place in March 1989 and restocking was initiated in April 1989. Matings of CBA/j♀ × CBA♂ mice following this time showed no significant difference from the previous groups in terms of the proportions of females mated, the apparent time to successful preg-

Table 4
Embryo implantation and incidence of fetal resorption rate by year

Year matings assayed	No. of CBA/j female mice	Embryos per uterus	% Embryos resorbed
1985	16	6.5 ± 1.9**	20.4 ± 16.6
1986	46	7.9 ± 2.4	25.5 ± 17.2
1987	51	8.1 ± 1.3	22.9 ± 10.5
1988	52	8.7 ± 2.4	20.7 ± 13.6
1989	21	8.0 ± 1.5	22.1 ± 13.9
1990	4	8.3 ± 0.5	21.2 ± 5.9
1991	100	9.5 ± 2.0**	19.5 ± 12.1
1992	8	9.0 ± 0.5	25.1 ± 10.1
Infected mice 1985–1988	165	8.1 ± 2.1	22.7 ± 14.1
SPF mice 1989–1992	133	9.2 ± 1.9	20.4 ± 12.2

Data are presented as means and standard deviations. See legend to Table 1 for general methodology. Mice mated in 1989 prior to the March 1989 decontamination are included in the data for the year 1988 which were presumably exposed to the endemic pathogens described in the text. Specific pathogen free (SPF) mice which were obtained and used after April 1989 were averaged with the data for the year of 1989. The implantation rates and incidence of embryo loss for each year were analyzed by one-way ANOVA. When the year groups were compared by the Tukey-Kramer multiple range method, the data for both 1985 and 1991 were significantly different from the other years at $P < 0.01$ (**).

nancy (data not shown) and the incidence of early embryo loss (Table 4). There was a significant improvement in the number of implanted embryos in these matings when the data for 1985, 1986, 1987 and 1988 (8.1 ± 2.1) were compared with the data acquired since 1989 (9.2 ± 1.9) but the incidence of embryo loss was identical (Table 4).

4. Discussion

The results of this study indicate that the pathogen exposure status of experimental mice with respect to viral pathogens, has no measurable effect on their reproductive performance and embryo survival. The only difference appears to be a slightly higher implantation frequency since decontamination. To some extent this counters the argument that an exogenous pathogen related environmental factor could have been responsible for the elevated embryonic losses in the CBA/j♀ × DBA/2♂ matings (Hamilton and Hamilton, 1987; Croy and Summerlee, 1990). The sources of bedding, food and water were the same, the quality of ventilation air was the same and the staff were the same while the cage cleaning detergents and cleaning routines were virtually identical. The higher incidence of embryo loss in the CBA/j♀ × DBA/2♂ matings must therefore be an endogenous genetic characteristic of this strain combination.

The effects of viral infection on early embryo losses are not well known but could either increase or decrease embryo loss. It is known that coronaviruses (including MHV) are intensely immunosuppressive and highly infectious (Casebolt et al., 1987; Lamontagne and Jolicoeur, 1991). Suppression of T-cell function is maximal during the active stages of disease within the first 2 weeks post infection but post-viral immune suppression can also persist for 3–4 weeks post infection (Casebolt et al., 1987; Lamontagne and Jolicoeur, 1991). Possible mechanisms for viral modulation of embryo survival could include the following: (1) the 'immunotrophism' hypothesis postulated by Wegmann, which states that the development of the fetal trophoblast is dependent on the presence of maternal cytokines, infers that the impairment of maternal T-cell function by MHV infection should dramatically diminish fetal trophoblast growth and prevent normal placental development leading to increased early fetal loss (Wegmann, 1987), (2) the local induction of high levels of cytokines by MHV could have a direct embryotoxic effect (Hill et al., 1987), (3) MHV infection may increase fetal resorptions due to the activation of cell mediated cytolytic activity by local cytokine (e.g. interferons) production, or (4) the virus could directly infect the fetal tissues causing embryo death, but conversely (5) MHV infection could also increase fetal acceptance by suppressing the maternal rejection response. Thus, viral infection could have negative or positive effects on embryo survival.

While this study has effectively eliminated a role for some of the specific murine viral pathogens in embryo loss, it does not exclude a role for other non-pathogenic commensal or opportunistic organisms which may be present in the normal flora of even healthy animals. A previous study to identify the involvement of environmental pathogens (viruses) in embryo loss, failed to identify a specific agent (Hamilton and Hamilton, 1987). However, the animals in that study were also stated to be infected with multiple pathogens and, even though a distinction was made between a room containing mice which were infected with only MHV and a room infected with Sendai and MHV, one must consider that a rigorous SPF regimen was not used and that the effects seen could have been due to the actual active disease status of the mice under study. In fact, the authors postulate that the low incidence of embryo loss in the SPF room was due to a non-pathogenic immunostimulatory factor which could simulate the protective effect of vaccination with BALB/c spleen cells, and not due to the absence of an abortogenic pathogen. Immunosuppression following a recent infection by MHV could have this effect on embryo survival. Therefore, our data and the data of Hamilton and Hamilton (1987) appear to exclude a role for exogenous viral pathogens in initiating embryo loss. Rather, the underlying defect in early pregnancy appears to be endogenous since it is unaltered by exposure to most exogenous viral pathogens.

Whether the actual maternal effector mechanism responsible for embryo loss is immunological or endocrine is unknown, but the underlying determinant must be genetically based and paternally expressed. This is clearly indicated by the observation that DBA/2j♂ induce resorption, while BALB/c♂ and many other inbred males do not. Furthermore, this trait segregates in back-cross experiments in a Mendelian fashion confirming an endogenous genetic basis (Bobe and Kiger, 1989). Abortion may not be due to classical immune recognition since paternal alloantigenic differences in the MHC have been shown in humans and experimental animals to be a positive factor for reproductive success. Therefore one is inevitably led to the more intriguing question of how MHC identical parents and inbred animals successfully gestate viable offspring if MHC identity is a negative factor. The elucidation of these and other questions will have to await further investigations. Thus, chance and the availability of the CBA/j♀ by DBA/2j♂ mating model provided a unique opportunity to study some of the exogenous factors potentially causing early embryo losses. This study showed that exogenous pathogen related factors do not contribute significantly to early post-implantation embryo losses.

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