Profile of Endometriosis in the Aging Female Rhesus Monkey

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Background. A retrospective analysis was conducted on necropsy records from a large rhesus monkey colony to evaluate the age-related prevalence of endometriosis.

Methods. A total of 314 records collected over a 15-year period were analyzed, yielding 66 monkeys with histologically verified endometriosis and 248 control subjects.

Results. The analyses demonstrated that the incidence of endometriosis increases progressively across the life span, ultimately impacting 21-45% of aged monkeys over 20 years of age.

Conclusions. Because mild disease is often not diagnosed premortem, the endocrine and immune sequelae of endometriosis may be a potential confound in even nonreproductive research with aging primates. Prior research-related events influence the occurrence and severity of endometriosis in these long-lived animals, and specifically could have contributed to the high prevalence of endometriosis in this particular monkey colony.

OLDER monkeys and aging humans experience many of the same ailments and, thus, monkeys have been used to model a wide array of age-related conditions, including metabolic, neural, and neoplastic diseases (1). Of particular relevance, the monkey is also an appropriate model for several gynecological conditions and the process of reproductive senescence (2,3). The following study evaluated the prevalence and manifestations of endometriosis, a disease involving the ectopic growth of endometrial implants outside the uterus, across the life span of the rhesus monkey. Many previous papers have reported that endometriosis occurs naturally in those higher primate species that menstruate, and it can also be induced experimentally by surgical procedures, such as hysterotomy (4-7).

Support for the natural occurrence of disease in these monkeys can be found by considering the epidemiology of endometriosis across the Primate Order. Although endometriosis has been described in many primate species, including the great apes, it occurs most commonly in the closely related African and Asian monkeys (Subfamily Cercopithecinae). For example, mild endometriosis has been observed in 8–17% of recently captured baboon females (8). Moreover, when baboons are maintained in captivity, disease incidence increases along with a progressive development of more severe endometriosis (9). In part, this morbidity appears to be associated with prolonged periods of menstrual cycling without the intervening pregnancies that would occur naturally at 1- to 2-year intervals (9,10). Less is known about disease at the other end of the life span, when one might anticipate a lower incidence or remission of symptomatology with the onset of reproductive senescence and menopause at 24–26 years of age (2,11). This age-related question was considered in the following evaluation of endometriosis in both middle-aged and old monkeys. Our retrospective analysis of disease in a large female cohort indicated that endometriosis is an age-related disorder in the rhesus monkey.

A survey of the literature provided a second rationale for our study, which was to assess several earlier life events that might facilitate a predisposition for endometriosis in female monkeys. One major risk factor appears to be prior abdominal or uterine surgery. Our evaluation focused on the prior occurrence of either laparoscopic examinations or caesarian deliveries, two events that had occurred commonly in this particular colony of monkeys (12). A second risk factor considered in our evaluation was exogenous estrogen administration, because hormone treatments, especially estrogenic ones, may be associated with the onset of endometriosis (13,14). In addition, the female monkey's body weight was considered in the analyses because obesity alters estrogen metabolism and, thus, could be associated with this gynecological condition. This interest in risk factors was stimulated by evidence that other types of experiential events, including exposure to certain environmental toxicants, such as dioxin, and radiation can increase the incidence of endometriosis in monkeys (15-17).

MATERIALS AND METHODS

Subjects. The database was generated from an evaluation of necropsy records on adult female rhesus monkeys...
(Macaca mulatta) that died between 10 and 37 years of age. It reflects the normal mortality across a 15-year period in a large research colony of approximately 1200 monkeys maintained at the Wisconsin Regional Primate Research Center. All necropsies performed between 1981 and 1995 were screened initially, and 314 were selected for analysis after a review of the monkeys’ experimental histories. Records were excluded if the female had received an ovariecctomy/hysterectomy during adult life or had been exposed to exogenous hormones during fetal life. These 314 necropsy reports included 66 monkeys with a histologically verified diagnosis of endometriosis and 248 age-matched females without detectable endometriosis. The monkeys had died for one of three reasons: (a) due to natural aging, (b) humane euthanasia for an incurable disease, or (c) euthanasia at the end of a terminal research protocol.

Categorization of endometriosis. —Because the severity of disease varied across monkeys, endometriosis was rated on a 0–3 scale based on the number, character, and distribution sites of lesions: 0 reflecting no disease, 1 for mild disease, 2 for moderate disease, and 3 for severe disease. The schema roughly followed the guidelines of the revised American Fertility Society classification system (rAFS) for categorizing endometriosis in women (18). Mild endometriosis was scored when there were a small number of implants, often on the serosal surface of the reproductive tract (typically ovary, uterus, and oviducts), with a limited amount of fibrous adhesion. Moderate disease was classified when there were more fibrous adhesions in the para-ovarian region or parametrium, focal hemorrhages, and usually some involvement of the gastrointestinal tract. Severe endometriosis was indicated if there were multiple areas with fibrous adhesions — pelvic or widespread — and large pseudocysts filled with sanguinous fluid. In the severe category, there was sometimes widespread dissemination with massive involvement of the bowel. In some cases, it resulted in intestinal obstruction that became the direct cause of death or reason for euthanasia. In all cases, the original pathologist (H.U. and M.L.Z.) had confirmed the diagnosis of endometriosis — whether mild or severe — with microscopic analyses of endometrial glands and stroma.

Descriptive statistics and experiential histories. — Age at the time of death, suspected cause of death, and date of necropsy were recorded for all females. Two reviewers examined each record and scored the stage of endometriosis on the 0–3 scale at 0.5 intervals. In addition, the computerized records of the complete life history were evaluated for each female. The analysis focused primarily on the occurrence of prior surgical procedures, including laparoscopic examinations, caesarian-section deliveries, and any other abdominal surgery. In addition, the records for females with endometriosis were reviewed to determine whether they had been involved in a research protocol involving estrogenic treatments. To evaluate if obesity might influence the course and severity of disease, adult weight at 10 years of age, and the last weight prior to death were also calculated for females with endometriosis.

Data analysis. — The distribution of endometriosis from 10 to 30+ years of age was examined initially by a χ² analysis of five 5-year age blocks. Subsequent analyses focused on the 66 monkeys diagnosed with endometriosis, and primarily involved one- and three-factor analyses of variance to compare monkeys with mild, moderate, and severe disease.

RESULTS

Prevalence of endometriosis. — The percent of monkeys diagnosed with endometriosis increased progressively across the life span, rising markedly from middle-age to a peak of 45% between 25 and 29 years of age (Figure 1). When considered in 5-year age blocks, the age distribution differed significantly [χ² (4) = 18.53, p < .01]. Post hoc analysis of the age blocks indicated that fewer monkeys had endometriosis in the 10–14 year category, whereas more had endometriosis in the 25–29 age group than would be expected by chance. Among the 66 animals diagnosed with disease, endometriosis was indicated as the direct cause of death for 20 monkeys. In 12 of these cases, it led directly to their demise, and for the 8 others, the severity of disease necessitated humane euthanasia. For these 20 monkeys, death occurred at an average of 20.3 years. Based on the blind scoring of the necropsy records, they were rated as having severe endometriosis (mean rating score = 2.58).

Although this finding suggests that disease morbidity and

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Figure 1. Percent of female monkeys exhibiting endometriosis at the time of death, as determined by gross necropy and histopathology. Sample size for controls and affected females in each 5-year age category is indicated above the bars. Disease incidence increased significantly with age, especially after 20 years of age. Monkeys are typically classified as aged when over 20 years of age; menopause occurs at 24–26 years of age.
mortality were directly associated — that endometriosis resulted in a shorter life span — the overall data indicated otherwise. When all 66 monkeys with endometriosis were considered, including those that did not die from complications associated with endometriosis, affected females actually died at an older age than did the nonaffected controls [21.4 vs. 15.4 years of age, respectively \( F(1,312) = 14.13, p < .001 \)]. This statistic may seem confusing, but was due to the fact that the incidence of endometriosis increased with age, even though the severity of disease did not necessarily progress with age. The mean age at death did not differ significantly between monkeys with mild, moderate, or severe disease \( F(2,63) = 2.59, p < .08 \). Indeed, even this non-significant tendency was inversely related to age, because monkeys with severe endometriosis (rated between 2 and 3) tended to die at a slightly younger age (mean age at death: severe = 19.3 vs. mild/mod = 22.5 years of age). Mild and moderate disease was not lethal and was often first diagnosed postmortem in the older females.

**Staging of endometriosis.** — Based on the numerical rating scale, the 66 monkeys with endometriosis were classified as having either mild \( (n = 20) \), moderate \( (n = 16) \), or severe disease \( (n = 30) \). Figure 2 shows that the endometriosis was more disseminated in severe disease, and impacted the ovary, intestines, and spleen more commonly in those monkeys rated between 2 and 3 on the basis of multiple parameters. Bisecting the data for the 66 monkeys, based on whether each tissue site was or was not involved, indicated that disease was rated more severe if the ovary [2.2 vs. 1.7; \( F(1,64) = 4.19, p < .05 \)], intestines [2.4 vs. 1.5; \( F(1,64) = 16.40, p < .0001 \)], or spleen were compromised (2.9 vs. 1.9; \( F(1,64) = 5.17, p < .03 \)). In addition, endometriosis on the bowel and Douglas pouch was especially common in the monkeys with severe disease (9 of 30 cases).

While the staging of endometriosis was closely associated with the number of impacted sites, the distribution pattern of implants and cysts was not strongly correlated with age. Considering whether or not a particular organ was impacted in the analysis of variance (ANOVA) yielded only one statistically significant effect on the monkeys’ age at death. If there was intestinal involvement, monkeys tended to die at a younger age \( [19.5 ± 0.8 \text{ vs. } 23.2 ± 1.0; F(1,64) = 9.02, p < .004] \). This effect of intestinal involvement was evident across all three stages of disease and occurred even when the ovary was not compromised.

**Antecedent events.** — Examination of the experimental and clinical records for each animal indicated that most females with endometriosis had been exposed to a prior life event that might initiate or aggravate disease (Table 1). Affected females had been utilized more frequently in research protocols involving laparoscopic examinations \( F(3,310) = 4.09, p < .008 \), although a Scheffé post hoc analysis indicated that this difference in exam frequency was accounted for largely by the severe group. The mean difference in procedures between mild, moderate, and severe disease was not statistically significant, nor did the number of instances correlate directly with the severity rating \( (r = .09, \text{N.S.}) \). Similarly, 24% of the females with endometriosis had undergone at least one prior hysterectomy, typically in the context of a caesarian delivery, but the incidence did not differ significantly between the rating categories. Overall, the female monkeys had experienced a mean 1.1 (± 0.2) abdominal surgeries, including C-sections, C-sections (1 or 2) (% of females) 

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<th>Disease Status</th>
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**Figure 2.** Percent of females showing endometrial implants, cysts, and adhesions on ovaries, intestines, and spleen. Data are portrayed with respect to diagnosis of mild, moderate, or severe disease. Severe endometriosis often impacted the small and large intestines in the rhesus monkey.

| Table 1. Descriptive Statistics Summarizing the Total Lifetime Occurrence of Prior Events that Could Have Contributed to the Development of Endometriosis in Females with Mild, Moderate, and Severe Disease |
|---------------------------------|---------|---------|---------|---------|
|                                 | Controls | Mild     | Moderate | Severe  |
|                                 |         | (248)    | (20)     | (16)    | (30)    |
| Laparoscopic exams (total frequency) | 3.3 | 5.2 | 4.3 | 7.5 |
| Laparoscopic exams (1 or more, % of females) | 54 | 40 | 31 | 77 |
| Caesarian section (no.) | 0.5 | 0.2 | 0.3 | 0.3 |
| C-section (1 or 2) (% of females) | 23 | 15 | 31 | 23 |
| Estradiol treatment (% of females) | 26 | 40 | 31 | 67 |
| Number of offspring | 4.9 | 2.6 | 2.9 | 3.2 |
| Weight at 10 years (kg) | 6.6 | 6.5 | 7.3 | 7.3 |
| Last weight premortem (kg) | 6.5 | 5.6 | 6.4 | 6.4 |

**Notes:** Data are the mean (± SE). ND, not determined.
during their lives. A higher percent of monkeys with endometriosis had been used in prior research projects involving estradiol treatments than had the control subjects ($\chi^2 (3) = 10.62, p < .02$), but it too remained a marginal predictor of disease severity. Bisecting the disease ratings, based on whether a female had been administered exogenous estrogen ($n = 33$ of the 66), revealed only a trend for more severe endometriosis ($2.2 \pm .2$ vs. $1.8 \pm .2; F(1,64) = 3.54, p < .065$). Weight at 10 years of age was a good predictor of individual differences in adult size and correlated significantly with the final weight before death ($r_{adj} = .39, p < .002$), but it did not distinguish endometriosis categories or correlate with age at death.

**DISCUSSION**

This retrospective evaluation of necropsy records clearly demonstrates the high prevalence of endometriosis in the rhesus monkey and the progressive increase in incidence with age. These data confirm earlier papers reporting that spontaneous disease is most common in monkeys between 10 and 20 years (19) and extend the findings into old age. Because gerontological research is typically conducted with monkeys over 20 years of age, the high incidence impacting 21–45% of aged subjects is a potential concern. At this older age, mild and moderate disease is often not diagnosed until postmortem and, thus, could influence the outcomes of even nonreproductive research unbeknownst to the investigator. There was a trend for a lower incidence in female monkeys over 30 years of age that were presumably postmenopausal, and it now remains to be determined whether this was a reflection of the earlier mortality in severe cases or due to a regression of symptomatology. It should also be acknowledged that this most elderly group of females is somewhat unique, having survived about 10 years longer than the normal life span in nature.

This age profile of disease in the monkey differs from the demographic pattern in women, in whom an adolescent onset is common (20,21), but the prevalence is not far above levels reported for some human populations, such as in women seen in infertility clinics or with menstrual dysfunction (22,23). The prevalence in the monkeys could have even been a little higher than we report here, because it is likely that some small or deep lesions associated with mild disease were not detected at necropsy (24). The actual presentation of endometriosis did have certain monkey-specific characteristics. These female monkeys frequently had lesions, cysts, and adhesions involving the ovary and intestines (67% and 73%, respectively, of the severe cases), which compares favorably with the distribution pattern reported for radiation-exposed monkeys (86% ovarian and 66% intestinal) (14). In contrast, human disease is more frequently found at sites close to the fallopian tubes, with lower gastro-intestinal involvement, and ovarian implants have been reported in only 10–24% of cases (23).

Our analyses indicated that the distribution of sites was not significantly affected by age, only by severity of disease. It is certainly likely that aspects of endometriosis are progressive, but the specifics need to be resolved in the monkey, and it has also remained controversial in women (25,26). The higher occurrence of mild disease in older animals could be interpreted as indicating that many female monkeys do not show progressive disease spread or a deepening penetration of implants with age. Alternatively, it could reflect a late disease onset, or regression of symptoms in old age. The highest mortality due to endometriosis occurred between 15 and 22 years of age, largely due to compromised gastro-intestinal function. Clinically, it sometimes become manifest as anorexia, constipation, and weight loss in the monkey. In contrast, when mild endometriosis occurs, it appears to be relatively benign and does not interfere with fertility in the monkey (27). In our study, mild endometriosis at least did not appear to affect the cumulative number of infants born across the life span.

D’Hooghe et al. (8,9) have argued that although endometriosis may be a spontaneous and natural disease of the monkey, certain experimental procedures and long-term housing in captive conditions without breeding may aggravate disease incidence and course. Our database was generated from a monkey colony that had been utilized extensively in reproduction research and, thus, had experienced many surgical and hormonal procedures purported to increase the likelihood of endometriosis. While these analyses could not prove that any single procedure was the major risk factor, a more sophisticated statistical analysis and larger database might delineate the relative contribution of each procedure. A recent study on baboons indicates that repeated laparoscopy exams over 32 months is sufficient in this species (28). However, because many female monkeys in our population had experienced multiple risk factors, it was difficult to discern the influence of each procedure, and it is likely that the effect was summative. In support of this conjecture, it is noteworthy that we have found endometriosis to be virtually nonexistent in a subset of this female population maintained independently at another facility since 1971 (unpublished data). These genetically related females and their daughters have been utilized only to breed infants at the Harlow Primate Laboratory and, therefore, they have annual pregnancies with only a few months of menstrual cycling each year. The absence of disease in regularly bred females lends support to the view that recurrent, retrograde menstrual flow may be a requisite event to seed endometriosis (29). It may also be that pregnancies are preventative or therapeutic for endometriosis in the monkey (30), although some disagree (27). What is known is that when rhesus monkeys do not breed and are exposed to certain risk factors, such as radiation, environmental toxicants (e.g., dioxin), or hysterotomy, the incidence of endometriosis can increase up to 58–100% (15,17).

In conclusion, the rhesus monkey, along with other species in this family of African and Asian monkeys, provides an excellent animal model for both spontaneous and experimentally induced endometriosis. Because disease first becomes manifest in middle-age and can be aggravated by a number of factors, it offers a unique opportunity for the study of life events and age-related disease. In particular, the high occurrence in menopausal monkeys over 25 years of age would allow one to evaluate the role of ovarian hormones in the maintenance and expression of disease. A better understanding of the physiology of endometriosis is critical for all gerontological research relying on the use of elderly female monkeys.
ENDOMETRIOSIS AND AGING

ACKNOWLEDGMENTS

This research was enabled by the Endometriosis Association, Milwaukee, WI, which provides salary support for S.E.R. C.L.C. receives partial salary support from National Institute on Mental Health grant MH41569; C.L.C., H.U., and M.L.Z. are supported by the Wisconsin Regional Primate Research Center (RR00167).

Special acknowledgments are due Dr. G. Lubach and Ms. H. LeRoy for their help in summarizing and analyzing this large data set.

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