Development of Spontaneous Endometriosis in Baboons

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Objective: **To determine the incidence of spontaneous endometriosis over a 32-month period in baboons with initially normal pelves.**

Methods: **In this observational longitudinal study, which was performed at the Institute of Primate Research, Nairobi (Kenya), 24 baboons with laparoscopically confirmed normal pelves underwent 67 serial laparoscopies (mean 2.8 ± 1.9, median 3, range 1–6) after 1–3 months (n = 8), 4–6 months (n = 11), 7–9 months (n = 9), 10–12 months (n = 17), 13–15 months (n = 6), 16–18 months (n = 4), 19–21 months (n = 3), 22–24 months (n = 4), 25–27 months (n = 1), and 30–32 months (n = 4). During each laparoscopy, the pelvis was examined for the presence of endometriosis. The number, size, and type of endometriotic implants were noted on a pelvic map, and the endometriosis score and stage were tabulated according to the revised classification of the American Fertility Society. Taking into account the variable length of follow-up, we used life-table analysis to calculate the cumulative incidence of endometriosis.**

Results: **The cumulative incidence of minimal endometriosis (proven by histology) was 64% up to 32 months of follow-up. The eight baboons that developed proven endometriosis were followed over longer periods of time and had undergone more laparoscopies than the animals that did not develop the condition.**

Conclusion: **There is a high incidence of minimal endometriosis in baboons, which increases with the time of follow-up and the number of repeat laparoscopies.** ***(Obstet Gynecol 1996;88:462–6)***

Nonhuman primates are the only animals that have menstrual cycles and develop endometriosis spontaneously. The baboon can be considered an interesting model to study laparoscopic appearances,[1] histology,[2] pathogenesis,[3] and spontaneous evolution[4] of endometriosis. Our preliminary study[4] showed that, after 10–12 months, endometriosis was present in seven of ten animals with previously normal pelves, an unexpectedly high incidence; however, only a limited number of animals was studied, the period of follow-up was short, and there was no histologic confirmation of endometriosis. Therefore, we performed the present study to document the incidence of histologically proven endometriosis in a larger group of baboons during a longer follow-up period.

Materials and Methods

During the study period (July 1990 through March 1993), 127 female baboons of proven fertility in the wild (with pendulous nipples as sign of at least one breastfeeding period[1]) underwent initial diagnostic laparoscopies at the Institute of Primate Research, Nairobi, Kenya, after approval by the Institute Scientific Resources Evaluation and Review Committee. One hundred three animals were excluded from this study because of the presence of endometriosis or pelvic adhesions at initial laparoscopy, irregular cycle, occurrence of pregnancy, medical treatment, laparotomy, or assignment to another research project during the study period. We included 24 baboons, 20 *Papio anubis* and four *Papio cynocephalus*, based on the following criteria: normal pelvis, regular cycle, no medical or surgical intervention during the study except for the follow-up laparoscopies, and no pregnancy during study period. Fourteen of the baboons were new and ten had been included in a preliminary study in which follow-up was only 10–12 months and there was no histologic diagnosis of endometriosis. The mean (± standard deviation [SD]) weight of animals was 11.3 ± 3.1 kg. Their age was unknown, but they had been in the colony for a median of 19 months (range 6–91). Four of the baboons

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had had hysterotomies for termination of pregnancy 16 ± 21 months (median 7, range 4–48) before this study began. None had delivered spontaneously in captivity. The end of the study was well defined (March 1993), but newly captured baboons entered the study at different times, resulting in a variable length of follow-up and variable number of laparoscopies for individual baboons.

Following a baseline laparoscopy confirming normal pelves in all 24 animals, 67 serial laparoscopies (mean 2.8 ± 1.9, median 3 per animal, range 1–6) were performed after 1–3 months (n = 8), 4–6 months (n = 11), 7–9 months (n = 9), 10–12 months (n = 17), 13–15 months (n = 6), 16–18 months (n = 4), 19–21 months (n = 3), 22–24 months (n = 4), 25–27 months (n = 1), and 30–32 months (n = 4). We used anesthesia, laparoscopy, and endometriosis screening techniques as described previously.[1] All baseline and follow-up laparoscopies were performed by the same investigator. Clinical endometriosis was defined as the presence of typical and/or subtle lesions. Lesions were classified as typical, subtle, or suspicious, according to the appearance of endometriosis in women.[5] Typical lesions were blue-black puckered lesions or blue-black cysts. Subtle lesions included red lesions (vesicular, polypoid, hemorrhagic, red flame-like) and white lesions (serous or clear vesicles, white plaques with or without clear or white vesicles). Peritoneal orange zones (excluding hemorrhagic or yellow-brown lesions) or irregular blood vessel patterns were considered as suspicious lesions.

During serial laparoscopies, the evolution of individual lesions (type, number, size, and surface area) were recorded on a pelvic map. Because most implants were circular, the surface area for each lesion was calculated using the formula πr^2 and expressed in mm^2. Endometriosis was scored according to the revised American Fertility Society classification system.[6]

At the last follow-up laparoscopy, 18 of the 115 endometriotic lesions were biopsied in eight of the ten baboons that had developed clinical endometriosis. The biopsies were fixed in 10% phosphate-buffered formalin, dehydrated, and embedded in paraffin. Serial sections (4 μm) through the entire lesions were stained with hematoxylin and eosin and histologically studied. Endometriosis was diagnosed using pathologic criteria of the human disease (presence of endometrial glands together with stroma at ectopic pelvic sites).

For statistical analysis, we used the two-tailed independent t test. Because the time of follow-up (interval between initial and last laparoscopy) varied between 6 and 32 months, we used life-table analysis[7–9] to calculate the cumulative incidence of endometriosis. Data are presented as mean ± SD, median, and range.

Results

Ten of 24 (42%) baboons developed clinical endometriosis with laparoscopic appearance and pelvic localization, as described previously.[7,10] Subtle and typical endometriotic implants were found in nine and four animals, respectively. The total number of endometriotic lesions (the combination of all lesions present in individual baboons at the last laparoscopy) was 115 (median 4.5, range one to 40), including six typical implants (5%), 108 subtle lesions (94%: 72 white or clear vesicles, 33 white nodules or white plaques, three red vesicles or polypoid lesions) and one suspicious implant. Biopsies were taken from 18 lesions (three typical, 14 subtle, one suspicious) in eight of the ten baboons that developed endometriosis. In the other two, biopsies were omitted by error. The histologic confirmation rate was 100% for the eight biopsied baboons and 50% for the 18 biopsied lesions (33, 50, and 100% for typical, subtle, and suspicious lesions, respectively).

Taking into account the variable length of follow-up for the 24 baboons, the cumulative incidence of endometriosis based on clinical diagnosis was 40% after 12 months and 70% up to 32 months of follow-up. The cumulative incidence based on histologic diagnosis was 29 and 64% after 12 months and up to 32 months, respectively (Figure 1, Table 1). Baboons with histologically proven endometriosis (n = 8) had significantly longer periods of follow-up (P = .005; mean 24 ± 4 months, median 22, range 20–31) and a significantly higher number of serial laparoscopies (P = .009; mean 4.2 ± 1.9, median 4.5, range 1–6) when compared with primates that did not develop endometriosis (mean 13 ± 8 months, median 10, range 5–31, and mean 2.2 ± 1.5 laparoscopies, median 1.5, range 1–5, respectively).

During follow-up, the number (Figure 2) and surface area (data not shown) of endometriotic lesions increased, due predominantly to the appearance of subtle implants (94% of all lesions). Although fluctuations were observed in both number (Figure 2) and surface area (data not shown) of implants, disappearance of endometriosis was not observed in any animal. Progression of endometriosis beyond stage I disease was not found.

Discussion

We used the life-table method of analysis[9–11] to determine the cumulative incidence of endometriosis in baboons with initially normal pelves that would develop endometriosis at any given period of follow-up. Life-table analysis has been reported to be the method of choice for the determination of the cumulative

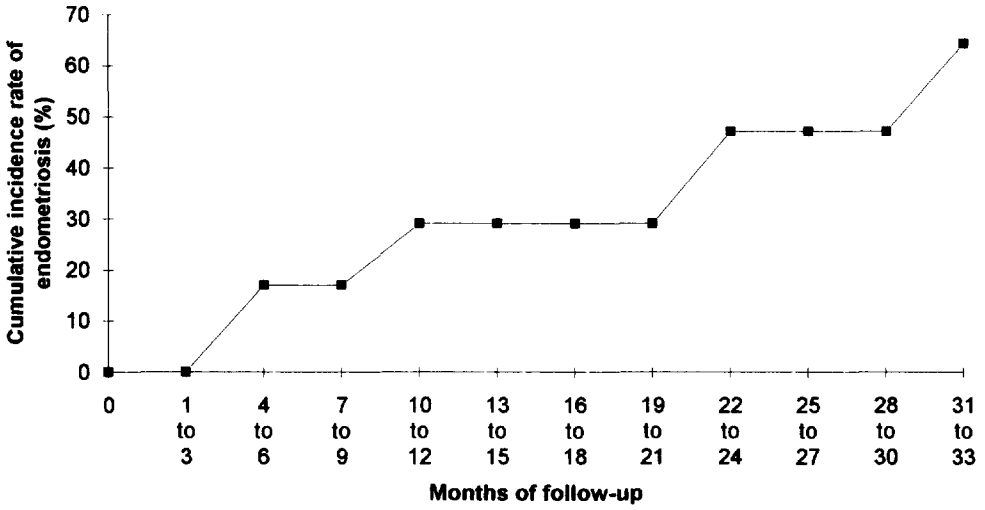

Figure 1. Cumulative incidence of spontaneous endometriosis in 24 baboons with initially normal pelves over a 32-month period. The cumulative incidence of histologically proven disease was 29 and 64% after 12 and 30 months, respectively.

pregnancy rate in fertility studies,[9] because there is adjustment for variability in the duration of patient follow-up.[9–11] This study satisfied three requirements of life-table analysis[10]: specific entry point (baseline laparoscopy), specific outcome date (date of detected endometriosis, date when study ended) and variable length of follow-up (6–30 months). All baboons were followed until their last laparoscopy, on which censoring was based. For some, the last laparoscopy was the one during which a diagnosis of endometriosis was made and later substantiated on pathologic examination. All other animals were considered to be followed until the last laparoscopy before the end of the study period. If, at the last laparoscopy before the ending of the study, the baboon had no evidence of endometriosis, then the baboon was censored at that point and considered to have no evidence of endometriosis during the period from enrollment until the last laparoscopy. During each 3-month interval, calculations were based only on the at-risk population, eg, the baboons that had not developed endometriosis but were still followed. The cumulative incidence of histologically proven endometriosis in baboons was 29% at 12 months and 64% at 32 months of follow-up. It cannot be excluded that our findings regarding long-term follow-up are attributable to chance, because all conclusions regarding follow-up after 12 months were based on two baboons with endometriosis. The proportion (42%) that actually developed endometriosis was lower than the cumulative incidence because bias was induced by the variable length of follow-up; the proportion of baboons that develop endometriosis can be invariably increased by lengthening the follow-up period, and, conversely, can be artificially lowered with a brief interval of follow-up. Life-table analysis with calculation of cumulative incidence of endometriosis corrected for this variable follow-up period.[7–9]

Table 1. Incidence of Histologically Proven Endometriosis by Length of Follow-up

Months follow-up	No. of baboons	No. of baboons with endometriosis	No. of baboons censored
0	24	0	0
1–3	24	0	0
4–6	24	4	2
7–9	18	0	4
10–12	14	2	6
13–15	6	0	1
16–18	5	0	1
19–21	4	0	0
22–24	4	1	0
25–27	3	0	0
28–30	3	0	0
31–33	3	1	2

The cumulative incidence of endometriosis in this study was much higher than the maximum cumulative rate of recurrent or persistent disease of 19%, achieved in the fifth postoperative year in 359 women with pelvic pain treated by laparoscopic surgery.[10] It is possible that the incidence in our study was higher because all initial and follow-up laparoscopies were carried out by the same investigator who screened specifically for subtle lesions in the whole pelvis, and because only one lesion with histologically proven ectopic endometrium was required to diagnose endometriosis. Baboons can be considered good models for the study of endometriosis in women for the following reasons: nearly identical reproductive anatomy and physiology, presence of peritoneal fluid, occurrence of retrograde menstruation, similar macroscopic and microscopic appearance, and

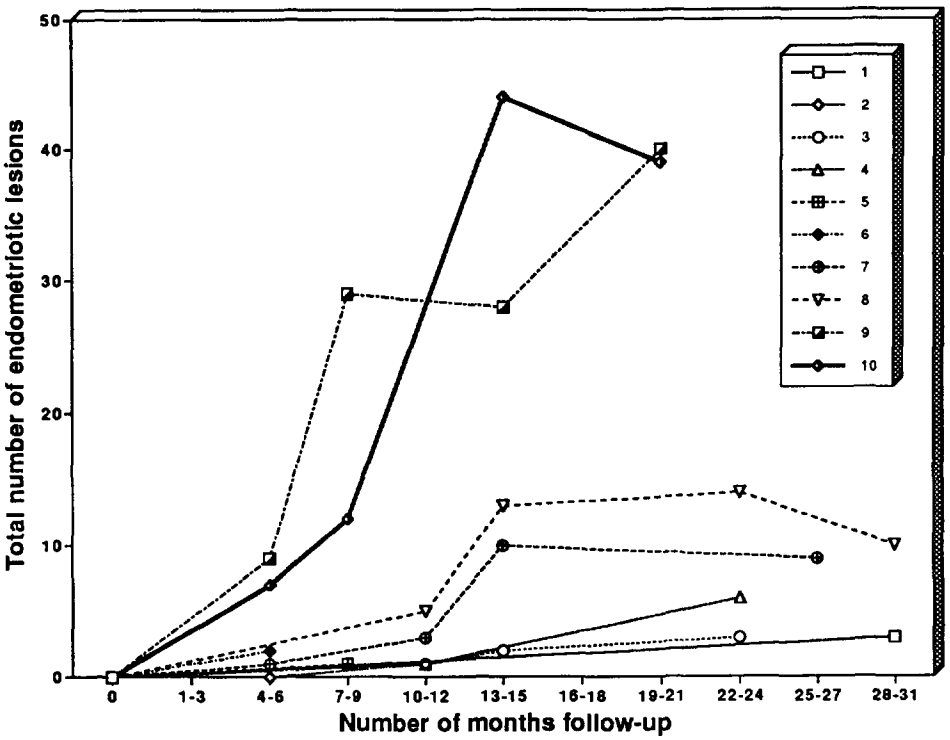

Figure 2. Incidence of endometriosis in 24 baboons: total number of endometriotic lesions for each of the ten baboons that developed clinical endometriosis (each symbol represents one baboon).

comparable localization of spontaneous endometriosis.[1,2]

The period of follow-up was appreciably longer in the group that developed endometriosis than in those that did not develop the disease, implying that the incidence of endometriosis increases with the increase in length of follow-up period. This finding is supported by the observation that the prevalence of spontaneous minimal endometriosis in baboons increases with the duration of captivity.[11] Several observations suggest that minimal endometriosis may be a common asymptomatic condition in menstruating primates: the results of the present study, the high prevalence (31%) of spontaneous minimal endometriosis in baboons that have spent more than 2 years in captivity,[11] the high prevalence (20–40%) of mostly minimal to mild endometriosis in asymptomatic women of proven fertility having laparoscopic tubal ligation,[12–13] and the normal monthly fecundity rates reported in both women[14] and baboons[15] with minimal endometriosis. The higher number of repeat laparoscopies in the baboons that developed endometriosis can be explained by the longer follow-up period in this group. Alternatively, it is possible that repeat laparoscopies favor the development of endometriosis, as hypothesized previously,[4] by local inflammation in the pelvic cavity with activation of immune cells and increased secretion of growth factors that may promote adhesion and implantation of ectopic endometrium, or by the induction of endometrial metaplasia of pelvic peritoneum.

Nearly all the new endometriotic implants in this study were subtle. These results confirm the high incidence of endometriosis after 12 months in baboons with initially normal pelves and support the concept that subtle lesions are young implants.[4,16]

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