Age as a determinant of reproductive success among captive female rhesus macaques (*Macaca mulatta*)

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Abstract

A retrospective analysis was performed on fertility outcomes among a colony of captive Indian rhesus monkeys. The analysis covered over 30 years and was based on 1443 females with a total of 11,453 pregnancies. Various determinants of fertility were assessed including birth rates, pregnancy loss, infant survival, interbirth intervals, and interval from last birth to death. Binary variables were analyzed with generalized linear models with random intercepts, while linear mixed models were used for analysis of continuous variables. Age of the dam was a significant factor in determining whether a pregnancy resulted in a birth and whether an infant survived the first 30 days with primiparous or older mothers being less likely to produce an infant surviving to that age. In contrast, sex proved to be the only significant factor in determining whether an infant lived to 1 year, with females being more likely to survive. The interval between births proved to be affected primarily by dam age, while the late death of an infant depressed the likelihood of an extended time interval between her last birth and her death. Overall, these results demonstrate that maternal age contributes significantly to a decline in fertility and older females can live relatively long periods following birth of their last infant.

Introduction

Rhesus macaques reproduce readily in captivity, although reproductive outcome is often determined by interactions between season, social status, nutritional status, body weight, and age. The onset of fertility in this species occurs between 42 and 48 months and, again, depends on various genetic and environmental factors. Consequently, animals give birth for the first time at about 4–5 years of age, with fertility subsequently peaking between 8 and 10 years, and a steady decline thereafter (Drickamer 1974, Anderson & Simpson 1979, Johnson & Kapsalis 1995). Since rhesus macaques are seasonal breeders, 65% of births in the colony under study occur in April–June.

The age at which reproductive senescence occurs in nonhuman primates is not well defined, although studies on the subject are emerging because of the interest in developing nonhuman models for studies of human menopause. Pavleka & Fedigan (1999) reported an age range for reproductive senescence of 14.5–32.7 years in Japanese macaques. Pigtail macaques experience a gradual fertility decline beginning at about 9 years, eventually reaching cessation at 19 years (Ha et al. 2000). Rhesus macaques, the most frequently used species for reproductive studies, reach reproductive senescence sometime between 10 and 24 years of age (Johnson & Kapsalis 1995).

Several reports have described the anatomical and physiological changes associated with age in rhesus macaques. Histological evidence has shown follicle depletion patterns in rhesus ovaries similar to those observed in the human ovary (Nichols et al. 2005). An increase in gonadotropin releasing hormone concentrations, associated with a reduction of estrogen at menopause, has also been reported in aged rhesus monkeys (Gore et al. 2004). Aging rhesus monkeys experience irregular menstrual cycles and long follicular phases due to delayed ovulation (Gilardi et al. 1997). Moreover, Schramm et al. (2002) found that as monkeys age, serum follicle stimulating hormone (FSH) levels increase, peak estradiol levels during hyperstimulation decrease and oocyte retrieval numbers are lower when...
compared with younger animals, which is consistent with what is commonly seen in older women.

Nevertheless, while rhesus monkeys experience similar reproductive changes as women, there is a significant difference in the timing of these events (Bellino & Wise 2002), which might, in part, stem from the seasonal breeding pattern of rhesus monkeys. Indeed, Shideler et al. (2001) monitored rhesus monkeys for a complete year and found that menstrual cycling in older females frequently ceased during the nonbreeding season. They also reported that the monkeys in their study did not exhibit a rise in baseline FSH during the transition to menopause, although that has been reported in perimenopausal women (Santoro et al. 1996).

While population studies of both captive (van Wagenen 1972, Pavleka & Fedigan 1999, Ha et al. 2000) and free ranging (Johnson & Kapsalis 1995) rhesus monkey colonies, as well as clinical observations (Nichols et al. 2005, Schramm et al. 2002), have confirmed age-related changes in reproductive patterns, there is still considerable ambiguity about the exact time at which reproduction stops entirely, as an event that is separate from mortality.

This study was, therefore, designed to determine the effects of various parameters on fertility with particular consideration of the effects of age on reproductive efficiency.

Materials and Methods

Animals

Reproductive records of a colony Indian of rhesus macaques (Macaca mulatta) were analyzed covering the years 1970–2006. The data set contained records of 1443 females with a total of 11453 pregnancies. All animals were experimentally naïve, although some animals may have been used in observational behavioral studies, which would have had no bearing on their fertility. Because of the time period covered by this paper, it is possible that some animals came from the founding stock (i.e. were wild caught). Over the years, other monkeys have been added to the colony from other primate center colonies to increase genetic diversity, but these would be considered captive or colony born.

As the objective of this study was to assess possible changes in reproductive patterns during a female’s lifetime, only those animals with a minimum of five pregnancies were included in these analyses. It was assumed that females with fewer pregnancies had most likely not lived long enough to develop age-related changes in fertility.

All rhesus monkeys were housed in large outside corrals in social groups with access to food and water ad libitum. Supplemental fruits and forage were provided weekly. Initially, each of the 22 half-acre corrals contained on average 45 adult females and 5 adult males. Animals were monitored daily by trained animal care staff. Sick or injured animals were removed and brought to a designated clinic for evaluation by the attending veterinarian. All animals received semi-annual health examinations, which included tuberculin skin testing and anthelmintic treatment. Pregnancy loss was determined only by physical evidence, i.e. after finding a late stage fetus and/or a placenta and when these could be unequivocally assigned to a specific female.

Statistical analysis

General approach

Much of this study is focused on the relation of birth outcomes to various covariates. Three separate analyses were performed to identify factors associated with different pregnancy outcomes: a) factors related to a pregnancy resulting in an aborted fetus, b) factors related to an early death (within the first 30 days) given a live birth, and c) factors related to a late infant death (infants dying between 1 and 12 months of age) given survival past 1 month. Additional analyses were performed to identify factors associated with the length of the interbirth intervals (IBI), the occurrence of extended IBI, the length of the menopausal period, and the occurrence of extended menopausal periods. For binary response models, logistic regression or logistic regression with random intercepts was used. For models with continuous responses, linear regression or linear mixed models were used.

Models for birth outcomes

Most of this study centers on covariates associated with various binary birth outcomes. In general, these observations are likely to be correlated. Pregnancy outcomes from a single dam probably have an inherent correlation because of dam-specific attributes, and in addition, past outcomes for a given dam may influence current outcomes. Consequently, suitable models can be complex and difficult to implement computationally.

Random effects models

For the analysis of the effects of ordinary covariates on binary birth outcome variables, logistic regression models with random intercepts for each dam were used. As is standard, it was assumed that the population of dam-specific intercepts is normally distributed with zero mean and unknown variance. These models were implemented through PROC GENMOD of SAS, using adaptive Gaussian quadrature with 63 quadrature points for each dam.
Extended random effects models

For two binary birth outcome variables, aborted fetus and early infant death, covariates included the immediate previous values of these birth outcome variables. For the analysis, the Markov assumption was made that the distribution of each of these two outcome variables for a given pregnancy, conditional on the entire past history of the dam, can be written as a logistic linear function of the ordinary covariates, the values of these two birth outcome variables for the immediate previous pregnancy, and a dam-specific random intercept. A primiparous indicator variable was included to accommodate the first pregnancy for each dam. It was further assumed that the two dam-specific random intercept variables in these two birth outcome models are uncorrelated. To implement a full maximum likelihood analysis, the likelihood functions for these two binary birth outcome variables could then be maximized separately, using the same SAS procedures as for the random effects models above. While there may have been a nominal loss of efficiency of estimation in choosing to maximize the two likelihoods separately, the dataset is quite large. It is unlikely that the estimation of the main effects was affected in any important way, and there has been a considerable gain in computational facility. Nonetheless, to confirm the validity of the effects obtained from these models, the data were analyzed second time with partly conditional mean models, which use generalized estimating equations to relate population proportions to covariates but allow dependence on selected past outcomes and history (Pepe & Couper 1997). The results obtained by this latter method agreed qualitatively with what was obtained from the random effects models, although they were not numerically identical, as the models are not intended to be entirely equivalent.

The factors that were entered into these models are, Abort (=1 if stillbirth, 0 otherwise), EarlyDth (=1 if live birth and death within first 30 days), LateDth (=1 if survives 30 days but dies before 1 year), Weaned (=1 if survives 1 year), DamAge (=dam age in years), Female (=1 if baby is female), Male (=1 if baby male), PrimDam (=1 if primiparous dam), PreIBI (=dam age at current birth minus dam age at previous birth), PostIBI (=dam age at next birth minus dam age at current birth), PreLongBI (=1 if PreIBI exceeds 1.5 years), PostLongBI (=1 if PostIBI exceeds 1.5 years), PAbort (=1 if previous fetus aborted), PEarlyDth (=1 if previous birth resulted in an early death) and LaIBI (=ln[0.5(PreIBI + PostIBI)]).

To understand the reasons for having indicator variables PreLongBI and PostLongBI, it is helpful to review the histogram of birth intervals IBI (Fig. 1), which shows a primary peak at about 1 year, and secondary and tertiary peaks at 2 and 3 years, and so on. This is caused by the seasonal breeding cycles of these animals; consequently the indicator variables PreLongBI and PostLongBI, suggest a missed previous or subsequent breeding season. The limit of 1.5 years serves to divide the group of IBIs peaking around 1 year from those peaking around later years.

Linear mixed models

In some analyses, the response variables were continuous, not binary, and so a linear or linear mixed model was used. In particular, to study the effect of DamAge on the length of IBI, the response variable chosen was LaIBI, the natural logarithm of the average of PreIBI and PostIBI. The variable LaIBI deserves explanation. From the definitions of PreIBI and PostIBI, it is apparent that current DamAge occurs in PreIBI with a positive sign and in PostIBI with a negative sign; therefore, the correlation of DamAge with PreIBI has an artificial positive bias, while the correlation of DamAge with PostIBI has an artificial negative bias. To eliminate or at least mitigate these artificial effects the interbirth response was based on the average of PreIBI and PostIBI. Finally, to bring the response closer to normal the final response was taken to be LaIBI. For this analysis, a linear mixed model (random intercepts, grouped by dams) was then used and implemented through the procedure lme from the contributed R package nlme.

To determine the existence and nature of menopause, the variable time to death (TTD; time from birth of last infant to death of dam) was analyzed by least squares regression for dependence on DamAgeLB (=age of dam at last birth). To avoid censoring bias and yet restrict the dams to animals that had died by the end of the time during which these records had been generated, only the 878 dams born before March 31, 1984 were included in this particular analysis. In addition, logistic regression was used to analyze the probability of TTD > 4.5 years for dependence on appropriate covariates. The routines lm and glm were used from the MASS library of the statistical package R.
Results

Infant births

Each female had an average of 7.94 pregnancies with a range of 1–19 live births. The highest proportion of infants was produced by 7- to 10-year-old females. The distribution of live infants within each age of mothers is shown in Fig. 2.

Pregnancy losses

A total of 707 pregnancies failed in 541 females. Out of these, 78 had occurred during the first and 253 during the last pregnancy. The ages of the females at the time of fetal loss ranged from 2.3 to 26.4 years. An extended random effects model with binary response variable Abort was implemented and revealed that the likelihood of pregnancy loss increased with advancing dam age ($P<0.001$), having a PrimDam as a mother ($P<0.001$), a preceding birth interval in excess of 1.5 years ($P<0.001$), and an aborted pregnancy ($P<0.02$) or early infant loss ($P<0.01$) in the preceding pregnancy. Among PrimDams, age had an inverse effect with younger females being less likely to carry a fetus to term ($P=0.005$). The percentage of pregnancies that terminated ranged from 5.4% in 4-year-old to 22.7% in 24-year-old respectively (Fig. 3).

Infant survival

A total of 8536 infants survived to 1 year of age, of which 4358 were female and 4160 were male, while 18 were recorded as ‘undetermined’.

Survival to day 30

Of the infants that did not survive to 1 year of age, 27.1% died within the first 30 days. An extended random effects model with binary response variable EarlyDth was implemented for all live births. Interestingly, essentially the same factors associated with a pregnancy loss proved to be factors associated with an infant’s death during this period. Thus, the likelihood of dying within this period was significantly increased by greater dam age ($P<0.001$), but not quite by having the preceding birth interval exceed 1.5 years ($P=0.06$). Similarly, having a PrimDam as a mother ($P<0.001$) and having an aborted pregnancy ($P<0.001$) or early death ($P=0.005$) in the dam’s preceding pregnancy were significant factors in elevating the probability an infant died during this period (see also Fig. 4). Finally, the inverse age effect prevailed for PrimDams, with young PrimDams being less likely to have an infant survive to 30 days ($P=0.001$).

Survival to 1 year of age

Among male infants that survived the initial 30 days, 19.8% (1024/5184) died before reaching 1 year of age, while 12.9% of females (646/5004) died during this time (73 cases without gender identification). This gender effect proved to be statistically significant (random effects model grouped by dam, $P<0.001$). In contrast, none of the other factors contributed to death past 30 days.

Interbirth intervals

Both the effects of covariates on the length of IBI and the effects of covariates on the occurrences of PreLongBI and PostLongBI were considered. To analyze the effect of DamAge on the length of IBI and avoid spurious correlations with DamAge, the IBI response was based on the average of PreIBI and PostIBI. The distribution of average IBI across dam ages is shown in Fig. 5. A linear mixed effects model with response variable LaIBI (=natural log of the average IBI) was implemented for the data in which first and last pregnancies were omitted. This analysis showed DamAge to be a
significant factor in increasing LaIBI ($P<0.001$). The effects of the outcome of the preceding pregnancy on PostIBI and PostLongBI are illustrated in Table 1. The table illustrates that late death (LateDth) has the lowest proportion of extended PostIBIs. Note that IBIs are rounded off for each birth outcome category.

Factors affecting PostLongBI ($Z_{\text{PostIBI}} > 1.5$ years) were formally analyzed with a random effects logistic regression model (random intercept, grouped by dam) relating PostLongBI to various covariates. This analysis showed that DamAge ($P<0.001$) was a strong factor increasing the likelihood of PostIBI $>1.5$ years, while the late death of an infant (LateDth) was a strong factor ($P<0.001$) decreasing this same likelihood. The data considered for this analysis were the same as for LaIBI, namely all pregnancies other than first or last.

**Post-reproduction period**

The least squares linear regression analysis of the relationship between the age at last birth of a dam and her subsequent TTD indicated that as dams become older at time of their last birth, the time to their death increased ($P<0.001$). Table 2 classifies dams according to their age at last birth and their subsequent TTD.

The proportion of dams whose TTD exceeded 4.5 years was also analyzed. These proportions by age at last birth are shown in Table 3. The trend of Table 3 was formally analyzed with a logistic regression which further indicated that the probability of an extended menopausal period (TTD $>4.5$ years) increases with dam age at last birth ($P<0.001$). Note that both tables show an increase in the time between last birth and death at around 13 years of age.

**Discussion**


Females in the present analyses began producing infants at about 4 years of age and the proportion of infants produced by females within an age group increased steadily up to 10 years, after which it gradually declined until reproduction ceased altogether. This reproductive pattern is similar to what has been reported in captive pigtail macaques at the Washington National Primate Research Center (Ha et al. 2000), and for Japanese macaques in Texas (Pavleka & Fedigan 1999). Female macaques can continue to produce infants well into their twenties, after which they cease to be reproductively active (Johnson & Kapsalis 1995, Pavleka & Fedigan 1999, Ha et al. 2000). This decline and ultimate cessation of reproductive capabilities of female macaques resembles that is seen in most other mammals (Cohen 2004) and is recognized to be a separate event from somatic senescence and more attributable to a
change in reproductive status than a decline in overall body condition.

The factors that determine longevity among rhesus macaques are likely to be as varied as they are among other primate species. However, one important consideration is that rhesus macaque groups have stable linear dominance hierarchies in which females of one matriline outrank females of lower matrilines. It is likely that this social structure has some bearing on the fate of at least some of the females in a group. Indeed, in many captive primates, especially macaques, social stress has been found to influence the incidence or progression of diseases such as atherosclerosis (Williams et al. 1991, Shively & Clarkson 1994, Shively et al. 2000, Kaplan et al. 1996, 2002, Skantze et al. 1998), respiratory infections (Cohen et al. 1997, Cohen 1999) and depression (Shively et al. 1997a, 1997b, 2005). Moreover, the relationship between social status and social stress is particularly consistent among females, with an inverse relationship between status and stress (Kaplan et al. 1984, Saltzman et al. 1991, Shively et al. 1997a, 1997b).

One of the notable effects of aging in females in the present study was a concomitant increase in pregnancy losses. This might, at least in part, be due to a decline in oocyte and embryo quality or changes in the uterine environment. Indeed, work on oocytes and embryos of humans, mice and other animal species has shown that the aging of the female is associated with a number of characteristic abnormalities including chromosomal aneuploidies (Volarcik et al. 1998), increased DNA fragmentation (Fujino et al. 1996, Wu et al. 2000), a decreased ability to mature and extrude a polar body in vitro (Peluso et al. 1980, Hewitt & England 1998), increased oxidative damage (Tarin et al. 1996, 2002), and an increase in the incidence and degree of mitochondrial damage (Keefe et al. 1995). Evidence of age-related changes in the uterine environment has been more difficult to ascertain and attempts have largely relied on reciprocal embryo transfer experiments in mice, hamsters, and rabbits. Transfers of embryos obtained from young animals into older recipients have shown a marked decline in pregnancy rates in these species (Blaha 1964, Talbert & Krohn 1966, Adams 1979).

The present study also revealed that the interval between births increased with the age of the mother, as did the late loss of an infant, while the length of preceding birth interval or infant sex did not seem to have any effect. Similar conclusions were reached by Wilson et al. (1983). In contrast, Maestripieri (2001) observed longer birth interval after daughters, possibly due to increased maternal investment in female offspring, while shorter birth intervals have been found to be associated with male offspring (Simms & Simms 1982).

A study of Japanese macaques (Fedigan et al. 1986) reported a nonsignificant trend towards a lengthening of birth intervals in aging females, while an age-dependent linear increase in birth intervals has also been described in pigtail macaques (Ha et al. 2000).

The fact that birth intervals tend to increase with advancing age may be a reflection of irregular ovulation patterns in older monkeys (Nozaki et al. 1995, Gilardi et al. 1997), as well as the increased strain on older mothers raising infants. In a study of the population on Cayo Santiago, older females were much less likely to produce an infant if they had given birth the previous season (Campell & Gerald 2004).

While the present study did not reveal any effects of infant sex on subsequent birth intervals for either sex, the results did indicate a higher mortality rate for male infants when compared with females, a trend also found in other colonies (Drickamer 1974, Maestripieri 2001). It was surprising that the present study revealed that the age of the mother appears to be a determinant of infant survival only during the first 30 days, while it does not seem to affect survival during the subsequent 11 months. It is not clear whether this reflects pre- or postnatal developmental problems among infants of older dams or a reduced mothering ability or a combination of both.

In the present study, the time between last birth and death increased significantly for older mothers. Up to age 19, females that died generally did so within the time of an average birth interval, indicating that their deaths were not preceded by extended periods of infertility. Monkeys surviving past age 22, however, showed much greater gaps between the time of birth of their last infant and death, which ranged from 3 to 8 years. The lengths of these intervals, which are much longer than the length of rhesus infant dependence, suggest that this may be the point at which menopausal transition has occurred.

There are two primary hypotheses that have been developed to explain the origin of menopause (Austad 1994). The adaptive theory, or grandmother hypothesis, proposes that menopause evolved as the risks of reproduction by older females were outweighed by the benefits of contributions made by these females to the survival of children and grandchildren (Williams 1957, Peccei 1995, Stanley & Kirkwood 2001, Lahdenpara

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Table 3 Relationship between age at last birth and subsequent menopause.

<table>
<thead>
<tr>
<th>Age at last parturition (years)</th>
<th>N</th>
<th>Average TTD</th>
<th>Prop &gt; 4.5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–11</td>
<td>75</td>
<td>1.069</td>
<td>0.04</td>
</tr>
<tr>
<td>11–12</td>
<td>112</td>
<td>1.016</td>
<td>0.018</td>
</tr>
<tr>
<td>13–14</td>
<td>175</td>
<td>1.387</td>
<td>0.063</td>
</tr>
<tr>
<td>15–16</td>
<td>151</td>
<td>1.358</td>
<td>0.033</td>
</tr>
<tr>
<td>17–18</td>
<td>125</td>
<td>1.420</td>
<td>0.088</td>
</tr>
<tr>
<td>19–20</td>
<td>91</td>
<td>1.179</td>
<td>0.055</td>
</tr>
<tr>
<td>21–22</td>
<td>86</td>
<td>1.332</td>
<td>0.081</td>
</tr>
<tr>
<td>23+</td>
<td>63</td>
<td>2.098</td>
<td>0.175</td>
</tr>
</tbody>
</table>

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et al. 2004). The alternative theory suggests that menopause is the result of nonadaptive evolution, in which menopause is a mere artifact of increases in lifespan, which are brought on by environmental factors such as better nutrition. In this view, a long post-reproductive lifespan does not enhance overall survival of offspring. Support for the latter view comes from species such as the olive baboon, in which family structures do include prominent grandmother involvement. However, it has been pointed out that offspring do not seem to derive any particular advantage from this arrangement (Packer et al. 1998).

Evidence reported to date supports the idea that macaques go through physical changes associated with reproductive senescence, reminiscent of changes observed during human menopause. Studies involving ovarian, hormonal, and pituitary function of aging rhesus macaques (Hodgen et al. 1977, Gilardi et al. 1997, Gore et al. 2004, Nichols et al. 2005, Schramm et al. 2002) have consistently shown biological changes mirroring those known to occur in women and support the macaque as an adequate model of human menopause. In Japanese macaques, a decrease in ovarian control of gonadotropin levels has been reported in monkeys approximately at age 18 (Nozaki et al. 1995).

Overall, our study, as well as those by others, shows that fertility declines as females age, due, at least in part, to changing aspects of the reproductive system. Moreover, cessation of fertility can precede death by several years. Nevertheless, given the often conflicting results of reports on rhesus macaque reproduction, in general, further studies are clearly needed.

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