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Feasibility of Successfully Breeding Rhesus Macaques (*Macaca mulatta*) to Obtain Healthy Infants Year-Round

Robert T. Beck¹, Gabriele R. Lubach¹, Christopher L. Coe¹

¹Harlow Center for Biological Psychology, University of Wisconsin-Madison, Madison, WI 53715

Abstract

Rhesus monkeys are typically seasonal breeders, but can be induced to extend the timing of their mating and births under captive conditions. The following analyses evaluated the potential impact of extending their pregnancies and deliveries year-round. Birth records from a large breeding colony housed in an indoor facility with a constant 14-hr light/10-hr dark cycle were analyzed across 25 years to examine seasonal trends in monkeys that mated in one of two ways: spontaneous in social groups or with a scheduled, timed-mating protocol. The dates of delivery and birth weights for 2084 infants were used in these analyses. Younger nulliparous females mating in social groups evinced a clear seasonal peak when birthing their first infant. However, older females, both primiparous and multiparous, could be bred continuously, which enabled the birth of infants in every month of the year. Based on the live birth rate, infant birth weights, high survival rates, and the normal sex ratio of infants birthed year-round, there were no adverse effects of breeding rhesus monkeys in this way. The continuous availability of infant births can be very advantageous for many types of research programs.

Keywords

rhesus macaque; birth weight; seasonality; reproductive success; breeding

INTRODUCTION

Most monkey species are seasonal breeders with the timing of the mating and birth periods typically oriented by annual variation in rainfall and food availability (Gesquiere et al., 2018; Rawlins & Kessler, 1985; Vandenberg & Vessey, 1968; Xiang & Sayers, 2009). However, when living in areas outside of the equatorial zone or if relocated to captive facilities in temperate latitudes, an additional influence of day length often becomes evident (Coe, Smith, & Levine, 1985; Van Horn, 1980). Primate facilities located in the Southern Hemisphere have also found that animals relocated from the Northern Hemisphere or from north of the equator experience a 6-month switch in the mating period, providing further evidence that day length can influence breeding (Bielert & Vandenberg, 1981; Hartman, 1931). The saliency of photoperiod can induce a number of primate species, including

Corresponding author: Robert T. Beck, Harlow Center for Biological Psychology, University of Wisconsin-Madison, 22 N. Charter Street, Madison, WI 53715, rbeck3@wisc.edu, Fax: 608-262-6020.

DATA AVAILABILITY STATEMENT. Historical birth records will be shared with other researchers interested in primate reproduction and husbandry upon request.

squirrel monkeys and macaques, to become short day/long night breeders, with the onset of mating activity and conceptions often associated with decreasing day length (Lemos, Downs, Raitiere, & Urbanski, 2009; Michael & Bonsall, 1977; Wilson, Gordon, & Collins, 1982). In rhesus monkeys, the physiological significance of the light and dark periods can also be demonstrated experimentally by showing there is a more extended nocturnal secretion of elevated reproductive hormones if the night is lengthened from 8-to-16 hours in duration (Lemos et al., 2009).

The primary aims of the following experiment and analyses were to assess how readily rhesus monkeys that typically breed seasonally could be induced to breed continuously across the entire year when living in an indoor facility with a controlled photoperiod and stable food availability. The additional aim was to verify that this extension of reproductive and birthing activity could be sustained over 25 years without any adverse consequences to their overall reproductive success or to the health of infants born across all months of the year. In addition, the husbandry practices were designed to allow younger, maturing females to follow a seasonal birthing pattern for their first pregnancy and then to transition to a continuous scheduled mating program for subsequent pregnancies.

Macaques, including the rhesus and cynomolgus monkeys, have traditionally been the most popular primates to use in biomedical studies. For that reason, domesticated breeding colonies have been established at many research centers in the United States and in other countries. When allowed to breed spontaneously in outdoor enclosures or under semi-natural conditions, most macaque species typically have demarcated mating and birth periods that can be from 3–6 months in length (Bielert & Vandenberg, 1981; Phillippi-Falkenstein & Harrison, 2003; Vandenberg & Vessey, 1968). At these locations or when kept in social groups at zoos, conceptions typically occur in the Fall months as day length declines followed by a birth peak 5.5 months later in the Spring (Gordon, 1981; Nozaki, 1991). However, it is also possible to breed rhesus monkeys in a more controlled manner by tracking the female's menstrual cycle and then housing them with adult males during only the fertile ovulatory days (Blakley, Beamer, & Dukelow, 1981; Dunk, 2013; Hendrie et al., 1996). The following analysis examined the effectiveness of this type of timed-mating protocol across 25 years and determined whether it was possible to override the intrinsic propensity of rhesus monkeys to be seasonal breeders.

The husbandry strategy was designed to test the efficacy of permitting younger, nulliparous females to mate spontaneously and birth their first infant in social groups before being transitioned to a year-round, timed-mating protocol. This timed-mating protocol was predominately comprised of older primiparous and multiparous females who were bred by pairing with an adult male for a delimited number of days when their fertility was optimal, approximately 2 weeks after menses. These pairings, in older primiparous and multiparous females, appeared to be effective across the entire year, documenting in a tangible way that it was possible to generate infants beyond the typical birth season. However, the success of this approach had not been evaluated in a systematic manner prior to the current analyses. The aim was to determine if there might have been an influence on pregnancy outcomes as indexed by live birth rates, the weight of infants at birth, postnatal developmental success during nursing, or the sex ratio of female-to-male infants across the year. The findings from

this evaluation of 2084 births across 25 years indicate it is feasible to have successful pregnancies and healthy infants born during all months of the year, which can be very advantageous for certain research projects.

METHODS

Subjects

Data were obtained from breeding records of rhesus macaques (*Macaca mulatta*) spanning a 25-year period from 1991 to 2015 at the Harlow Primate Laboratory (HPL), a facility located at the University of Wisconsin-Madison. The HPL has maintained a large breeding colony of rhesus macaques since the 1960's starting with a founder population derived from India. The colony has been closed to outside animals for nearly 3 decades. Thus, all of the current adult animals were born at the HPL and reared in a similar manner. None had ever been housed outdoors under natural conditions. The following analysis was based on all 2084 pregnancies and births that occurred from 1991 to 2015. Husbandry and breeding procedures were approved by the Institutional Animal Care and Use Committee, complied with the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and adhered to the American Society of Primatologists' Principles for Ethical Treatment of Non-Human Primates.

Housing

The animal vivarium at the HPL is located within a 3-story, 31,538 square foot facility and is comprised of 28 rooms with standardized environmental conditions. There are flexible housing options for individual, dyadic, and small group caging, which includes runs and pen-housing for larger social groups. Cages are cleaned daily and sanitized at 2-week intervals. All monkeys are housed indoors with artificial lighting on a 14-hr light/10-hr dark schedule. The constant photoperiod is used deliberately with the aim of overriding the species tendency to breed seasonally. Rooms where mating occurs, specifically the pens used for spontaneous mating and the two rooms used for the timed-mating strategy, do not have windows that would permit exposure to seasonal variation in daylength. Many of the other housing rooms where the older females live do have translucent windows that provide exposure to natural light; however, ceiling fixtures still provide light for 14 h each day. Due to insufficient data on the exact housing locations of individuals within the facility over the past 25 years, we could not assess the possible influence of translucent light exposure on the females in the timed-mating protocol when not actively breeding. However, all the males used for the timed-mating protocol were housed in rooms without any exposure to natural light and all mating occurred in these rooms.

Exposure to seasonal variation in temperature was also limited by maintaining the ambient room temperature at a mean 21° C year-round. All monkeys were fed the same standard primate chow each day (Purina 5LIQ, PMI Nutrition International, St Louis, MO), which was supplemented with fresh produce and grains. They were provided with regular environmental enrichment consisting of manipulanda, chewable objects, music, television, and movable mirrors.

Infants were raised by their mothers for at least 6 to 7 months until they were weaned into small social groups of similar age. Juveniles and subadults remained in social groups, typically in larger cages or pens, until the birth of their first infant. The HPL also has some larger social groups that are usually mixed-aged and contain 1 or more adults. All animals are housed in large rooms where visual and vocal interactions can readily occur.

Reproductive Strategies

This study compared the success of the timed-mating protocol to the spontaneous mating that occurred naturally in social groups. Groups with post-pubertal subadult monkeys were the primary source of spontaneous births and these females were usually nulliparous. In addition, some mixed-aged groups containing adult animals also generated spontaneous births from multiparous females. Beyond this breeding activity in social groups, the majority of adult females were involved in the timed-mating protocol, which necessitated they be housed individually or in same-sex pairs away from adult males. These females were housed with males only when relocated to the cage of a breeder male during the fertile ovulatory days of their cycle. The pairs typically remained together for 4–7 days. If a female did not conceive on her first cycle, she was then paired again during her next cycle. The current colony has 16 adult breeder males and approximately 150 adult females involved in the timed-mating protocol and produces 80 or more infants annually. Due to the fact that most females have their first infant in the younger adult groups as they transition past menarche, the timed-mating protocol is used almost exclusively with older, multiparous females.

Over the last 3 decades, the timed-mating protocol has enabled the breeding program to extend the births of infants year-round, with the goal of lessening the influence of the seasonality that is evident at most outdoor facilities. The purpose of the following analysis was to assess the effectiveness of this husbandry approach, to examine its efficacy by identifying any potential adverse effect on infant birth weight and viability, and to verify that the tendency for seasonality had truly been overridden.

Breeding Records

Infant birth records for the 25 years from 1991 to 2015 were analyzed. Inclusion criteria for the birth weight analysis included full-term births and infant weights obtained within 1 week of delivery. Applying these inclusion criteria, 1198 infants were used for the analysis that focused specifically on birth weight. The stricter inclusion/exclusion criteria were applied only to the birth weight analysis, which required that infants were weighed within the first week after delivery; the larger set of 2084 infant births were used for the other analyses. In addition to infant birth weight, the two breeding strategies were compared by analyzing temporal trends in deliveries across the 25 years, looking at seasonal variation within a given year, live birth rates across years, and the sex ratio of female-to-male infants across months of the year.

Historical birth records will be shared with other researchers interested in primate reproduction and husbandry upon request.

Data Analytical Strategy

Descriptive summary statistics were generated initially to determine means and variance for all variables. For the statistical analyses, variables were organized into four 3-month periods (i.e., January-March, April-June, July-September, and October-December), referred to as seasonal quarters, for both breeding strategies. To analyze seasonal and temporal trends across years, the births were summed into five 5-year blocks or lustrums (i.e., 1991-1995, 1996-2000, 2001-2005, 2006-2010, and 2011-2015). The effect of each breeding strategy on birth weights, live birth rates, infant survival rates to three months of age, and the sex ratio of female/male infants were assessed with analyses of variance (ANOVA) for the entire 25 years with each seasonal quarter and lustrum considered as a factor with nested levels. The total number of births for each seasonal quarter within the 5 lustrums were then normalized with respect to the total number of infants born for each breeding strategy across the entire 25 years. Variation in the percentage of births within each seasonal quarter and lustrum, determined with respect to the total number of births, was evaluated with ANOVA. Independent sample *t* tests were also used to compare seasonal variation in birth frequencies between the two breeding strategies. The magnitude of the differences between conditions and variables was evaluated by calculating effect sizes, with a partial eta squared (η_p^2) > .14 and a Cohen's *d* > .8 considered to be large effect sizes.

RESULTS

Table 1 provides descriptive summary statistics for the rhesus monkeys used in this analysis. A total of 2084 infants were included, with 1579 monkeys born from the timed-mating strategy and 505 born after spontaneous mating in the social groups (Table 1). Due to the larger number of infants from the timed-mating strategy, the births in each season of each 5-year period (lustrum) were divided by the total number of infants from each type of breeding. These normalized percentiles were used to examine if there was significant seasonal variation in birthing across the year.

Significant differences in the trend for seasonality were found when comparing the distribution of births in 3-month seasonal quarters for the two mating strategies ($F(3, 160) = 20.14$, $p < .001$, $\eta_p^2 = .27$). Post hoc *t* tests indicated significance for the April through June ($t(36.32) = 5.12$, $p < .001$, $d = 1.45$) and October through December seasons ($t(32.38) = 5.24$, $p < .001$, $d = 1.48$) (Table 1). These two seasons corresponded to the peak and nadir of births after spontaneous mating by females living in the social groups. In contrast, the extent of the temporal differences between the two mating strategies did not reach significance for the transitional seasons from January through March ($t(48) = 1.02$, $p = .31$, $d = .29$) or July through September ($t(48) = 1.67$, $p = .10$, $d = .47$). Comparison of the relative percent of infants born in each season from the timed-mating and the spontaneous mating did not change significantly across lustrums ($F(4, 160) = 1.38$, $p = .24$, $\eta_p^2 = .03$): 1991-1995 ($t(38) = .77$, $p = .45$, $d = .24$), 1996-2000 ($t(28.87) = .78$, $p = .44$, $d = .25$), 2001-2005, ($t(26.79) = .79$, $p = .44$, $d = .25$), 2006-2010 ($t(38) = 1.39$, $p = .17$, $d = .44$), and 2011-2015 ($t(27.29) = .24$, $p = .82$, $d = .07$) (Table 1). This similarity in birth numbers over time verified the stability of the husbandry practices in this breeding program and that the reproductive

behavior and outcomes did not undergo significant change across the 25-year period used for this analysis.

The relative number of infants born in each season was organized into 5-year periods to examine the distribution of births for the two mating strategies. Figure 1 shows the relative percent of infants born in each season, illustrating the seasonal variation evident for spontaneous mating in social groups as compared to the more stable, continuous birthing for the timed-mating strategy.

In keeping with the main effects described above, the interaction between birth season, lustrum, and mating strategy was non-significant ($F(12, 160) = .93, p = .52, \eta_p^2 = .07$). The two-way interactions between birth season and lustrum were not significant ($F(12, 160) = 1.06, p = .40, \eta_p^2 = .07$). In addition, the two-way interaction between lustrum and mating strategy was not significant ($F(4, 160) = 1.38, p = .24, \eta_p^2 = .03$). However, the two-way interaction between birth season and mating strategy was significant ($F(3, 160) = 20.14, p < .001, \eta_p^2 = .27$).

Across the four seasons of each lustrum, the timed-mating strategy did not differ significantly ($F(19, 80) = 1.23, p = .26, \eta_p^2 = .23$), while birthing from the spontaneous mating strategy followed a seasonal pattern ($F(19, 80) = 6.62, p < .001, \eta_p^2 = .61$) (Fig. 1). Post hoc *t* tests confirmed that the spontaneous mating strategy resulted in a single birth peak in April-June that differed significantly from all other seasons: January-March ($t(38.44) = 6.72, p < .001, d = 1.90$), July-September ($t(44.31) = 4.99, p < .001, d = 1.41$), October-December ($t(27.02) = 9.00, p < .001, d = 2.55$). There was also a single nadir, with fewer births in October-December, which differed significantly from the 3 other seasons: January-March ($t(32.76) = 2.40, p = .02, d = .68$), July-September ($t(29.42) = 3.91, p = .001, d = 1.11$).

An examination of parity verified the expected results that most conceptions after spontaneous mating were in nulliparous females, who would become first time mothers. Given that our husbandry regimen then transitioned most older females to the timed-mating protocol, the difference in the percent of infants born to primiparous and multiparous females in each mating strategy was statistically significant. There were more primiparous births from spontaneous mating ($t(174.18) = 3.26, p = .001, d = .46$) and more multiparous births from the timed-mating strategy ($t(117.90) = 13.91, p < .001, d = 1.97$) (Table 1).

The relative number of primiparous and multiparous births in each season for both mating strategies was compared to examine seasonality (Fig. 2). Primiparous births varied significantly across seasons in both the timed-mating ($F(3, 80) = 4.26, p = .008, \eta_p^2 = .14$) and spontaneous mating strategies ($F(3, 80) = 27.26, p < .001, \eta_p^2 = .51$), while births from multiparous dams varied significantly only in the spontaneous mating strategy ($F(3, 80) = 11.67, p < .001, \eta_p^2 = .3$) but not in the timed-mating strategy ($F(3, 80) = 2.26, p = .09, \eta_p^2 = .08$).

Differences in delivery outcomes between the two mating strategies were compared to evaluate the reproductive success and efficacy of the two mating strategies (Table 2). There were no significant differences when comparing the number of successful pregnancies

resulting in live births to the number of stillbirths (i.e., the live birth rate) ($F(1, 2082) = .02$, $p = .89$, $\eta_p^2 < .001$), mean infant birth weights ($F(1, 1196) = 1.50$, $p = .22$, $\eta_p^2 = .001$), or the sex ratio of female/male infants ($F(1, 2072) = .09$, $p = .77$, $\eta_p^2 < .001$) across the 25 years (Table 2). Seasonal variation in the number of mating sessions required for a conception to occur with the timed-mating strategy was also evaluated. There was not a significant difference in fertility, as indexed by time to conceive, across the 4 seasons ($F(3, 522) = 1.69$, $p = .17$, $\eta_p^2 = 0.1$).

Birth weight did not differ significantly between the two mating strategies in any season: January-March ($F(1, 226) = .005$, $p = .95$, $\eta_p^2 < .001$), April-June ($F(1, 345) = 3.40$, $p = .07$, $\eta_p^2 = .01$), July-September ($F(1, 352) = .02$, $p = .88$, $\eta_p^2 < .001$) and October-December ($F(1, 267) = 1.27$, $p = .26$, $\eta_p^2 = .005$) (Table 2). In addition, the female/male sex ratio did not change seasonally for either mating strategy: January-March ($F(1, 371) = .25$, $p = .62$, $\eta_p^2 = .001$), April-June ($F(1, 666) = 1.28$, $p = .26$, $\eta_p^2 = .002$), July-September ($F(1, 610) = .00$, $p = 1.00$, $\eta_p^2 < .001$) and October-December ($F(1, 40.91) = 1.92$, $p = .17$, $\eta_p^2 = .004$) (Table 2). The live birth rate was high in both mating strategies, with live birth rates averaging 94% for the entire breeding program (Table 2). The live birth rate remained stably high for the timed-mating protocol across the years and was similar for both mating strategies in three seasons: January-March ($F(1, 372) = 1.14$, $p = .29$, $\eta_p^2 = .003$), April-June ($F(1, 671) = .35$, $p = .55$, $\eta_p^2 = .001$), July-September ($F(1, 611) = .07$, $p = .80$, $\eta_p^2 < .001$). However, there was a slightly lower live birth rate during October-December for the spontaneous mating protocol ($F(1, 422) = 5.19$, $p = .02$, $\eta_p^2 = .01$) when it seemed that deliveries out of phase with the seasonal peak were less likely to result in a live birth (Table 2).

Females in the timed-mating protocol were also more likely to successfully raise their infants to 3 months of age ($F(1, 2076) = 34.90$, $p < 0.001$, $\eta_p^2 = .02$), although the overall infant survival rates were high in both husbandry conditions (Table 2). Three-month survival was slightly higher for the timed-mating strategy (99%) when compared to the spontaneous mating strategy (95%) (Table 2). There was also a significant interaction between mating strategy and season in infant survival rates ($F(3, 2076) = 6.97$, $p < .001$, $\eta_p^2 = .01$) because it remained high and stable at 98-100% in the timed-mating protocol, whereas it fluctuated some across seasons for infants born in the spontaneously mating social groups (Table 2). The highest infant survival success rates after spontaneous mating were evident when conceptions had occurred in the fall and winter months, whereas the success rate declined some after late summer conceptions, which would have resulted in infants being born when many other females in the social groups were pregnant (Table 2).

DISCUSSION

This analysis has confirmed that the breeding of rhesus monkeys can be extended year-round without adverse consequences. A high live birth rate and healthy birth outcomes were sustained over several decades, enabling viable and thriving infants to be born in every month of the year. The capacity of the older multiparous females to cycle and conceive continuously was in marked contrast to the maintenance of a seasonal trend in the reproductive behavior of younger nulliparous and group-housed females that continued to

evince an annual birth peak. The husbandry and breeding program at our facility had been purposefully designed in this way to allow the younger adult females to mate spontaneously in social groups, providing an opportunity for first-time mothers to acquire parenting skills in the presence of others. Subsequent conceptions for the older adult females were planned and scheduled primarily with the timed-mating protocol, by housing them with breeder males for a delimited number of days only when fertile at mid-cycle.

The timing of menarche and first conceptions in seasonally breeding primates is typically synchronized with the mating and birth seasons of the adult animals (Drickamer, 1974; Pittet, Johnson, & Hinde, 2017). Thus, maturing menarcheal females usually become primigravida at 3.0, 4.0 or 5.0 years of age, coinciding with the corresponding mating and birth seasons of the adult monkeys. Under natural conditions, this timing is shaped by multiple zeitgebers, including behavioral cues (Kaufmann, 1965) and climatic conditions, especially annual variation in rainfall (Gesquiere et al., 2018; Rawlins & Kessler, 1985; Vandenberg & Vessey, 1968) and day length (Bielert & Vandenberg, 1981; Hartman, 1931). Intrinsic annual biorhythms have also been reported for both male and female rhesus monkeys (Lemos et al., 2009; Michael & Bonsall, 1977; Wilson, Gordon, & Collins, 1982). In a captive setting with controlled environmental conditions and stable diets, we and others have shown that an early or late timing of menarche for female rhesus is also strongly influenced by their rate of growth and body weight (Shirtcliff et al., 2004). Larger females reach menarche and conceive for the first time at a younger age than do smaller ones. Similarly, under natural conditions, pubertal age and the timing of reproduction in wild baboons was found to be affected by growth rates associated with the abundance or constraints of food availability (Gesquiere et al., 2018).

Over the last 50 years, improvements in nutrition and husbandry have led female monkeys in many breeding programs to grow faster and reach menarche at a younger age. This precociousness may have contributed in part to our finding that the live birth rate was lower in one seasonal quarter for the social groups, whereas it remained stably high for the older, multiparous females. In addition, another factor contributing to the lower live birth rate in one quarter for the social groups may be that it was related to asynchronous births coinciding with when the other females in the group were mating and becoming pregnant. Small et al. (1986) had reported previously that there can be a lower survival rate for infant rhesus monkeys if born outside the typical birth season in outdoor enclosures. The lower live birth rate of 84% in one season differed from the mean success rate of 94.8% in the other 3 seasons for the spontaneously mating groups. It also likely reflected the younger age and inexperience of mothers responding to their first delivery. In contrast, the primarily older, multiparous females in the timed mating program mostly had healthy pregnancies that successfully reached term with unassisted deliveries. Across both breeding strategies, the overall live birth rate of 94% is comparable with other research colonies that have live birth rates of 95% when monkeys are in large social groups (Schapiro & Bernacky, 2012) and 92% in animals bred with a similar timed-mating protocol (Wolf et al., 2004).

Our conception rate with the timed-mating protocol was especially efficacious when compared to previous reports of conception rates in group-housed animals where less than 50% of the adult females may give birth in some years (Anderson & Simpson, 1979).

Moreover, the high infant survival rates for the females in our husbandry program should also be highlighted when compared to free-ranging rhesus monkeys. A ten-year summary of free-ranging rhesus monkeys on La Parguera found that younger primiparous mothers successfully raised only 55-60% of their infants to 6 months of age while older multiparous females had a 90% infant survival rate (Drickamer et al., 1974) while a more established rhesus colony on Cayo Santiago had a 91-92% infant survival rate (Koford, 1966).

It was also particularly striking that the older female rhesus monkeys were able to so readily transition from the seasonal pattern evident for first pregnancies to the reproductive flexibility clearly evident in the multiparous dams in the timed-mating strategy. The capacity to conceive at any time of year was due to the fact that female rhesus monkeys can continue to have multiple menstrual cycles after weaning their prior infant until the next conception (Williams & Hodgen, 1982). Unlike some seasonally breeding primates that may stop cycling as other females around them become pregnant, female rhesus monkeys will continue to have ovulatory cycles, at least when kept in domesticated conditions (Seth, 2000). When living in semi-natural conditions, such as for the rhesus monkeys on Cayo Santiago, it does appear the reproductive activity is more synchronized across females; those that don't conceive in a particular mating season may then defer until the following year (Seth, 2000; Vandenbergh & Vessey, 1968). A similar menstrual or behavioral synchrony may be responsible for the seasonal pattern in first time pregnancies in the timed-mating strategy. Births from primiparous mothers in the timed-mating strategy were predominately a result of females who had not become pregnant while living in larger social groups, a unique circumstance that accounted for 143 out of the 2084 births. When housed with just one other female in our facility, the adult monkeys continued to have fertile cycles and could be repeatedly housed with an adult male until they conceived. With our timed-mating protocol, conceptions typically occur within 1–3 pairings with the breeder males and can be efficiently induced in any month of the year after the first pregnancy.

The current analysis specifically focused on whether this year-round breeding might be associated with any inadvertent problematic concerns over time. Based on the outcomes we analyzed, there were no negative effects: on the likelihood of a successful pregnancy outcomes, infant birth weights, infant survival during the early nursing phase, or the sex ratio of female-to-male infants born across the year. In fact, the reproductive success of this colony over a 25-year period was consistently equal to or above the levels reported for other facilities, including both indoor and outdoor breeding programs (Gagliardi et al., 2007; Hoffman et al., 2010; Hopper, Capozzi, & Newsome, 2008; Pittet et al., 2017).

It should be acknowledged that when comparing all births across the entire study, there appeared to be some variation in birth frequencies between two seasonal quarters with the timed-mating protocol, but these differences were not statistically significant. The effect was due to some differences in the number of animals bred in certain years in order to fulfill the planned needs for infants in research projects. This trend can be seen in Table 1, which shows that there were fewer animals bred with the timed-mating protocol during 1996-2005. Similarly, in Figure 1 it can be seen that a lower number of females were bred in January-March of 1991-1995.

Therefore, some of our conclusions do reflect unique aspects of our husbandry practices. Because it was already known that the reproductive physiology of primates can be strongly influenced by day length, all of the monkeys were purposefully exposed to a constant photoperiod year-round with an invariant 14 h light/10 h dark schedule. However, it is still not clear what factor continued to cue the nulliparous and group-housed females to evince a seasonal trend in conceptions and births given that these social groups were housed in a large room without windows. The room temperatures were also regulated year-round, and their diet and food availability did not change seasonally. Interestingly, as the females got older, we did not see a persistence of overt seasonal rhythmicity like the one reported after Japanese macaques were transferred from their natural habitat to a zoological setting (Schucchi, Torisi, D'Amato, Fuccillo, 1981). In addition, it should be acknowledged that the animals in our colony have been selected for their breeding potential. It is now comprised entirely of descendants from a founder population that was purposefully selected for health and breeding, which may have contributed to both their high reproductive success and behavioral plasticity to breed year-round. The colony has been closed for decades to preclude the introduction of new viral pathogens, which also means that the timing of their reproduction would not have been influenced by exposure to animals that had been raised outdoors with seasonal variation in weather.

Notwithstanding these considerations, the overall reproductive and birth success clearly demonstrate that the rhesus monkey has the capacity to produce infants year-round. Continuous birthing is obviously different than the reproductive pattern in outdoor colonies and semi-natural conditions where the reproductive behavior is strongly influenced by seasonal variation in day length and the mating period is usually coincident with decreasing daylight and the presence of cycling females (Bielert & Vandenberg, 1981; Hartman, 1931). At our facility, this inherent tendency toward seasonality was seen only in the younger females and those housed in groups. Otherwise, signs of an annual reproductive cycle were largely absent because the scheduled timed-mating protocol was based on monitoring the females' menstrual cycles, which could occur year-round.

The ability to generate infant monkeys throughout the year can be very advantageous for certain types of investigations. For example, it ensures the availability of new infants at every maturational stage year-round and allows for simultaneous comparisons of adult females that are cycling, pregnant, and nursing without having to conduct the evaluations at different times of the year when day length, temperature, and seasonally available foods may differ. In addition, in extreme situations where seasonal pregnancies might coincide with natural and human-made disasters (Black et al., 2017), it then becomes possible to have contingency plans and navigate the timing of conceptions to preclude inadvertent fetal exposures.

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Mated vs Spontaneous Births Over 25 Years

Figure 1.

Relative percent of rhesus monkey births across 25 years, comparing a timed-mating strategy to spontaneous mating that occurred in small social groups. Infant births were normalized according to breeding strategy by dividing the number of infants born in each seasonal quarter of each year by the total number of infants born during that 5-year period. The relative percent of rhesus monkeys born in each season and divided into five 5-year periods are displayed along with standard error for both mating strategies.

Parity and Mating

Figure 2.

Relative percent of births considering the potential interaction between parity and seasonality in a timed-mating strategy (A) and spontaneous mating that occurred in small social groups (B). Infant births were normalized for each breeding strategy by dividing the number of infants born in each seasonal quarter by the total number of infants born to primiparous and multiparous females from 1991-2015. Mean (S.E.) percent of births are portrayed separately for each mating strategy and illustrate the stronger trend for seasonality in primiparous females, whereas multiparous females birthed more similar numbers of infants year-round with time-mating.

Table 1.

Summary statistics for the breeding of rhesus monkeys via either a timed-mating strategy or spontaneous mating.

Variable	Timed-Mating	Spontaneous	P value ¶
Number of Infants	1579	505	
Seasons †‡			
January-March	18.9%	15.0%	NS
April-June	25.1%	54.7%	< 0.001
July-September	31.5%	23.0%	NS
October-December	24.5%	7.3%	< 0.001
5 Year Intervals †§			
1991-1995	20.6%	16.2%	NS
1996-2000	16.8%	21.6%	NS
2001-2005	17.4%	21.4%	NS
2006-2010	21.7%	16.0%	NS
2011-2015	23.6%	24.8%	NS
Parity			
Primiparous	143	289	< 0.01
Multiparous	1436	216	< 0.001

† Percentages reflect the number of infants born in each 3-month quarter divided by the total number for each mating strategy from 1991-2015.

‡ Percentages are the portion of infants born in each season.

§ Percentages are the portion of infants born in each 5-year lustrum.

¶ Birth frequencies were normalized according to the breeding strategy. ANOVA tests were used to compare overall differences; t tests were employed post hoc after significant main effects were found to determine the significance of pairwise comparisons.

Table 2.

Successful delivery outcomes and infant birth weights for rhesus monkeys bred with a timed-mating strategy compared to spontaneous mating in social groups over 25 years.

Variable		Timed-Mating	Spontaneous	P value [‡]
Mean Birth Weight (grams) [†]	Total	500	492	NS
	January-March	499	501	NS
	April-June	499	484	NS
	July-September	502	500	NS
	October-December	498	527	NS
Female/Male Ratio	Total	754/821	242/256	NS
	January-March	158/139	38/38	NS
	April-June	183/214	137/134	NS
	July-September	235/261	55/61	NS
	October-December	178/207	12/23	NS
Live Birth Rate [§]	Total	94%	94%	NS
	January-March	93%	96%	NS
	April-June	96%	95%	NS
	July-September	95%	94%	NS
	October-December	94%	84%	< .05
Infant Survival [¶]	Total	99%	95%	< .001
	January-March	100%	89%	< .001
	April-June	99%	96%	< .01
	July-September	98%	97%	NS
	October-December	100%	95%	< .001

[†]Weights analyzed only if obtained within the first week of delivery.

[‡]One-way ANOVAs used to verify significant differences.

[§]Percentages reflect infants born healthy and not stillborn

[¶]Percentages reflect infants that survived at least three months and exclude stillbirths already included in live birth rates.