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Fertility Following Extended Depo Provera Treatment

in Sooty Mangabeys (*Cercocebus atys*)

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Fertility Following Extended Depo Provera Treatment in Sooty Mangabeys (*Cercocebus atys*)

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in partial fulfillment of the requirements for the degree of
Master of Arts in Psychology
2013

ABSTRACT

Fertility Following Extended Depo Provera Treatment
in Sooty Mangabeys (*Cercocebus atys*)
By Maurand M. Cappelletti

The injectable contraceptive medroxyprogesterone acetate (depo provera, DMPA), a synthetic progestin that suppresses ovulation, is currently approved for human use in over 90 countries. DMPA is also widely used for the reproductive management of animal populations. Studies examining fertility after DMPA treatment in both human and nonhuman animals have focused on the resumption of ovulation after treatment cessation, but neglected potential long-term effects of DMPA exposure on successful reproduction. DMPA is frequently administered as a contraceptive agent for adolescent girls, however, the possibility of differential long-term fertility effects of DMPA exposure during adolescence have not been explored. We investigated the effects of extended DMPA treatment on the post-treatment fertility of a species of old world nonhuman primate, the sooty mangabey (*Cercocebus atys*). Female sooty mangabeys (N=31) in a large breeding group at the Yerkes National Primate Research Center Field Station received DMPA treatment for between 4-8yr. At the time of first DMPA injection, females were either parous (N=14) or nulliparous (N=17), with nulliparous-treated females consisting of pubertal (N=10) and prepubertal adolescents (N=7). After cessation of DMPA treatment, nulliparous-treated females had a significantly higher incidence of stillbirth than did age-matched or experience-matched controls, whereas parous-treated females did not differ from matched controls. Looking exclusively within treated females, nulliparous-treated females placed on DMPA prepubertally had a significantly higher incidence of stillbirth than either pubertal nulliparous-treated females or parous-treated females. The majority of stillbirths to nulliparous-treated females included difficult and/or prolonged labor (dystocia) culminating in infant death. In humans, dystocia is associated with weak uterine contractions, and progestins are known to suppress uterine contractility. It is possible that exposure to elevated levels of progestin throughout puberty, a critical period of uterine development, permanently reduced uterine contractility for females placed on DMPA in early adolescence. These results indicate that the post-treatment effects of chronic DMPA exposure vary with the developmental timing of treatment onset, and raise concerns about the use of DMPA as a contraceptive for adolescent girls.

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INTRODUCTION

The synthetic progestin medroxyprogesterone acetate (depo provera, DMPA) is a 3-month injectable contraceptive that is currently approved for human use in over 90 countries worldwide (Khoiny, 1996). In the United States, DMPA has represented an increasingly popular form of hormonal contraceptive since its approval by the Food and Drug Administration in 1992, and today over 11 million women in the United States have used DMPA (Mosher & Jones, 2010). Due to its ease of administration and long-acting effects, DMPA is also a popular contraceptive option for the reproductive management of both captive and free-ranging animal populations, especially for captive colonies of nonhuman primates (Kirkpatrick & Turner, 1991). Return of fertility following DMPA treatment is of great concern to both users and prescribing physicians, and of particular importance in situations where DMPA is being used to reproductively manage populations of threatened or endangered animals. However, studies examining fertility after DMPA treatment in both human and nonhuman animals have predominantly focused on resumption of ovulation after treatment cessation, and neglected potential long-term effects of DMPA exposure on successful reproduction.

DMPA protects against pregnancy by acting at the level of the pituitary and hypothalamus to suppress ovulation. While progesterone and estradiol naturally fluctuate across the menstrual cycle, women using DMPA are exposed to consistently high levels of progestin which suppresses both the rise in estradiol across the follicular phase and the resulting mid-cycle surge in luteinizing hormone that triggers ovulation (Rivera et al., 1999). Studies of human DMPA users have reported that ovulation consistently returns following the cessation of DMPA treatment, although there is considerable individual

variation in latency to recovery (Fotherby et al., 1980; Schwallie & Assenzo, 1974). Several factors have been suggested to influence latency to return of fertility following cessation of DMPA treatment, including reproductive experience prior to treatment (Pardthaisong et al., 1980), total drug exposure (Garzes-Flores at al., 1985), and weight (Lan et al., 1984), however studies examining the relationships between these factors and latency to return of fertility have been generally inconclusive.

While there have been no studies explicitly investigating the effects of DMPA treatment on long-term reproductive capacity, two studies have examined the effects of exposure to a similar synthetic progestin, melengestrol acetate (MGA), on future infant survival in species of nonhuman primates. Unlike DMPA, MGA is administered as a subcutaneous implant, however both contraceptives protect against pregnancy via the same mechanism. Both DMPA and MGA are widely used for the reproductive management of captive nonhuman primates, although, unlike DMPA, MGA is not approved for human use. Wood et al. 2001 examined reproduction after MGA implant expiration or removal in golden lion tamarins (*Leontopithecus rosalia*), and reported a significantly higher stillbirth and infant mortality rate for females who had been exposed to MGA as compared to controls, however this difference was only significant for females whose MGA implants had been exhausted but were not surgically removed. The "empty" MGA implants could have been releasing low levels of MGA during pregnancy, making the results of this study difficult to interpret. De Vleeschouwer et al. (2000) examined pregnancy outcomes after MGA exposure in golden-headed lion tamarins (*Leontopithecus chrysomelas*), but limited their analysis to females whose implants had been surgically removed, and reported that females who had been exposed to MGA

showed a significantly higher rate of stillbirth after MGA treatment as compared to before treatment. However, treated females were also several years older after MGA exposure, and the effects of maternal age on stillbirth were not investigated. Nonetheless, both of these studies raise the need for further investigation into the effects of synthetic progestin exposure on future infant survival in nonhuman primates.

The present study examined return of fertility, reproductive output, and pregnancy outcomes after extended DMPA treatment in a species of old world primate, the sooty mangabey (*Cercocebus atys*). Sooty mangabeys are menstrual primates with an average menstrual cycle length that falls within the normal human range (Hadidian & Bernstein, 1979), and patterns of hormonal fluctuation across the menstrual cycle similar to those in humans (Aidara et al., 1991). Sooty mangabeys therefore represent an appropriate animal model for the study of processes relevant to human reproductive endocrinology. In the fall of 2001, all adult female sooty mangabeys living in a large social group at the Yerkes National Primate Research Center (YNPRC) Field Station were placed on DMPA to eliminate reproduction. Pre-adult females in the group were subsequently placed on DMPA around the time they reached puberty. All females in the group continued receiving DMPA treatment until the fall of 2009, and all females had stopped receiving treatment by the spring of 2010, at which point breeding in the group was allowed to resume.

Females treated with DMPA were either parous adults or nulliparous adolescents at the time of treatment onset, which allowed for investigation of the differential fertility effects of DMPA exposure during discrete stages of reproductive development. In the United States, DMPA is frequently administered as a contraceptive agent for adolescent

girls, and many clinicians recommend DMPA over oral contraceptives for adolescent patients given that DMPA is both highly effective and long-acting, decreasing the opportunity for user error and unintended pregnancy (Cromer et al., 1998; Khoiny, 1996; Tolaymat & Kaunitz, 2007). There is some evidence suggesting that DMPA may differentially affect adolescents, as adolescents have an increased rate of bone-density loss on DMPA treatment as compared to adult users (Cromer et al., 1996, Scholes et al. 2002). However, the relationship between stage in reproductive development at DMPA treatment onset and effects on future fertility have not been explored.

METHODS

Treated Subjects

DMPA-Treated Subjects

Treated subjects were female sooty mangabeys (*cercocebus atys*, N=31) living concurrently in one large multi-male/multi-female social group at the Yerkes National Primate Research Center (YNPRC) Field Station in Lawrenceville, GA. Housing consisted of a large outdoor enclosure (~230m^2) with an attached indoor quarters (~16.5m^2). At the time of DMPA treatment onset, treated subjects were either parous adults (N=14) or nulliparous adolescents (N=17), with nulliparous-treated subjects being further defined as either pubertal (N=10) or prepubertal (N=7). Female sooty mangabeys exhibit perineal swellings which fluctuate in size and turgescence across the menstrual cycle, with last day of maximal swelling being closely associated with ovulation (Aidara et al., 1981). Nulliparous-treated subjects were defined as prepubertal if they had never achieved maximal perineal swelling prior to first DMPA injection, indicating that they had never achieved ovulation. Conversely, nulliparous-treated subjects were defined as

pubertal if they had exhibited one menstrual cycle with a clear maximal swelling prior to first DMPA injection.

DMPA Treatment Administration

All treated subjects were treated with an injectable suspension of either original brand depo provera and/or medroxyprogesterone acetate (DMPA), obtained from various suppliers, with concentrations ranging from 100mg/ml to 200mg/ml. DMPA treatments were administered as intramuscular (IM) injections every 1-3 months by members of the YNPRC Colony Management Department staff. All treated subjects were placed on treatment for colony population management, and not for health concerns. All treated subjects received their first DMPA injection between September 2001 and March 2007, and their final DMPA injection between October 2009 and April 2010. Parous-treated subjects were older at the time of their first and last injection, were on treatment longer, and received higher total dosage of DMPA than did nulliparous-treated subjects (see Table 2).

Injection dosage varied both between treated subjects and between injections for a given treated subject, with a minimum of 40mg and a maximum of 300mg. Injection dosage varied to compensate for individual differences in body weight, and individual differences in rate of drug metabolism, in an effort to achieve consistent suppression of ovulation. Ovulation was continuously suppressed across the treatment period for 28 out of 31 treated subjects. Three treated subjects did not receive one of their DMPA injections soon enough to maintain suppression of ovulation, and become pregnant during the treatment period. These three females received no further DMPA injections during gestation and/or lactation, and after their infants were weaned DMPA treatment was

resumed. One nulliparous pubertal treated subject was the product of one of these three unintended pregnancies during the treatment period. While this subject was born of a mother exposed to DMPA, it is unlikely that this female was exposed to DMPA prenatally or while nursing, as her mother received no DMPA injections during gestation and/or lactation.

Reproductive Sampling Period

The reproductive sampling period was defined as the period of time over which reproduction was followed for each subject. Reproduction was followed for all treated subject for several years starting from the day of their first ovulation after their final DMPA injection (M=1.97±0.64 years). The sampling period for all treated subjects fell between January of 2010 and October of 2012.

Controls

All adult female sooty mangabeys in the YNPRC Field Station breeding colony were placed on DMPA treatment starting in October of 2001, therefore control females were retrospectively selected from the colony's historical records before the onset of DMPA treatment.

Age-Matched Controls

To control for effects of maternal age on reproductive output and pregnancy outcomes, an age-matched control (N=31) was selected from historical records for each treated subject. The reproduction of each age-matched control was retrospectively followed from the day when the control would have been that same age as was her matched treated subject on her first day of reproductive sampling (e.g. if a treated subject was 3500 days old on the first day of her reproductive sampling period, then the

reproduction of her age-matched control was retrospectively followed from the day in the past when the control would also have been 3500 days old). All age-matched controls had given birth at least once prior to the onset of their reproductive sampling period. The sampling period for all age-matched controls fell between December of 1983 and September of 1996.

Experience-Matched Controls

To control for effects of reproductive experience on reproductive output and pregnancy outcomes, an experience-matched control (N=17) was selected from historical records for each nulliparous-treated subject. The reproduction of each experience-matched control was retrospectively followed from the day of her first ever ovulation, therefore all experience-matched controls were nulliparous adolescents at the time of reproductive sampling period onset. The sampling period for all experience-matched controls fell between February 1989 and July of 1996. The experience-matched controls were significantly younger (M=1220.88±39.46 days) than the nulliparous-treated subjects at sampling period onset (M=3504.53±103.30 days, $t(32)=20.65$, $p<0.001$).

Group Size

Average group size across reproductive sampling period was lower for treated subjects (M=40.93±0.91) than for age-matched (M=85.37±28.98, $t(60)=8.74$, $p<0.001$) and experience-matched controls (M=98.87±7.5, $t(46)=40.67$, $p<0.001$). During their reproductive sampling period, all experience-matched controls, and 26 out of 31 age-matched controls, had been housed in the same enclosure as were the treated subjects. Five age-matched controls had been housed in a second, similar enclosure still at the YNPRC Field Station.

Data Collection

Clinical, caging, and reproductive histories for all subjects were retrieved from the YNPRC computerized animal records database (Animal Records System). Perineal swelling and early abortion data were retrieved from records kept by the YNRPC Colony Management Department.

Perineal Swelling Data Collection

Trained observers in the YNPRC Colony Management Department visually inspected the perineal areas of each sooty mangabey female in the YNPRC breeding colony five days per week, and rated swelling size on a scale from 0-4, with 0 indicating no swelling and 4 indicating maximal swelling. Observers also recorded any occurrence of post-conception swelling and/or implantation bleeding, established visual markers of specific pregnancy time-points in sooty mangabeys (Gordon et al., 1991). Conception dates were retrospectively calculated via backwards counting from the day of post-conception swelling and/or implantation bleeding. Two observers collected all perineal swelling data used in this study, and inter-observer reliabilities were performed. Inter-observer reliably was 85% agreement on swelling size ratings. A consistent perineal swelling data collection protocol was used for all subjects. For the purposes of this study, last day of maximal perineal swelling during a given menstrual cycle was used as a proxy for ovulation, following an established procedure for estimating ovulation in sooty mangabeys (Aidara, 1981).

Early Abortion Data Collection

All early abortion data were collected by the same two trained observers who collected all perineal swelling data used in this study. An early abortion was determined

to have occurred if a female was observed passing blood and/or tissue vaginally during a confirmed pregnancy, after which she resumed showing cyclic perineal swelling. Pregnancy was confirmed either via ultrasound, or via observation of a post-conception swelling and subsequent implantation bleeding. Early abortion data were only collected as part of YNPRC sooty mangabey colony management protocol after 2009, so abortion data were available for treated subjects, but not for age-matched or experience-matched controls.

Following Reproduction

Return of Fertility

Latency to return of fertility was followed for each treated subject after their final DMPA injection. Latency to return of fertility was measured by both latency to resumption of ovulation (defined as the number of days from final DMPA injection to first subsequent ovulation) and latency to first conception (defined as the number of days from final DMPA injection to first subsequent conception).

Reproductive Output

Reproductive output was measured for all subjects during their reproductive sampling period, and defined as total number of births across total number of days of reproductive opportunity. A given day was considered a day of reproductive opportunity if the subject spent the entire day in the group with access to at least one adult male. Any day that a subject spent out of the group was still considered a day of reproductive opportunity if the subject was already pregnant at the time. For treated subjects, days of reproductive opportunity during the sampling period varied between 203 and 954 days. Reproductive output for each age-matched and experience-matched control was

examined over the same number of days of reproductive opportunity as their matched treated subjects.

Pregnancy Outcomes

Pregnancy outcomes were followed for all treated subjects during the reproductive sampling period. Pregnancy outcomes were categorized as either positive or negative, and then further classified by type based on the definitions in Table 3. Definitions for stillbirth, early abortion, and perinatal death were consistent with those used by the Center for Disease Control (CDC) for classifying pregnancy outcomes. The incidence of each type of pregnancy outcome over total births during the sampling period was compared between treated subjects and matched controls. All treated subjects had 1-3 births during the sampling period, thus some treated subjects contributed more births towards total births during the sampling period than did others. To account for the overrepresentation of certain treated subjects in total births during the sampling period, pregnancy outcomes for each age-matched and experience-matched control were followed for the same number of births as their matched treated subject (e.g. if a treated subject had one birth during the sampling period then her matched control was followed for one birth). Thus the number of births contributed by each treated subject towards total births was mirrored in the age-matched and experience-matched controls. To further control for the overrepresentation of certain treated subjects in total births during the sampling period, pregnancy outcomes were examined exclusively for first births during the sampling period for both treated subjects and matched controls.

Statistical Analyses

All analyses used SPSS software (version 20.0.0, IBM Corp., Somers, NY). All tests were two-tailed, and were considered significant with a p value less than 0.05. Differences between parous and nulliparous-treated subjects in latency to resumption of ovulation and latency to first conception after final DMPA injection were analyzed with an independent samples t-tests for equality of means. Multiple linear regression models were used to examine the relationship between reproductive experience prior to treatment, weight at last treatment, total drug exposure, and both latency to resumption of ovulation and latency to first conception. Differences in reproductive output between groups were analyzed with independent samples t-tests for equality of means. Differences in the proportion of negative pregnancy outcomes between groups were analyzed with the Fisher's Exact Test (FET). The phi coefficient, a pearson product moment correlation coefficient that indicates the magnitude of association between two binary categorical variables, was used as a measure of effect size for all FET analyses. A phi coefficient of 0.10-0.30 was considered small, 0.30-0.50 was considered moderate, and greater than 0.50 was considered large, following conventions for interpretation of effect size in the behavioral sciences (Cohen, 1988). When attempting to balance the risk of type one versus type two error in our analyses, we decided that, given the exploratory nature of the present investigation, and the fact that negative pregnancy outcomes are rare but highly significant events, the risk of type two error was more serious. We therefore maintained an alpha level of 0.05, but did not systematically correct for multiple comparisons.

RESULTS

Return of Fertility

One parous-treated female failed to ovulate again within the study period after her final DMPA injection, and was excluded from the study. Individual latency to resumption of ovulation after final DMPA injection varied between 106 days and 317 days, and mean latency to resumption of ovulation did not differ between parous-treated subjects (M=185.86±14.91 days) and nulliparous-treated subjects (M=191.35±11.00 days, t(29)=0.30, p=0.76). Mean latency to conception after final DMPA injection also did not differ between parous-treated subjects (M=275.08±31.50 days) and nulliparous-treated subjects (M=260.06±12.22 days, t(28)=0.49, p=0.63). One parous-treated female failed to conceive again after her final DMPA injection and was excluded from analysis of latency to conception.

Combined, parity at first treatment, total drug exposure, and weight at last treatment did not significantly predict latency to resumption of ovulation after final DMPA injection, and explained only 2% of the variance in latency to resumption of ovulation (Table 4). Parity at first treatment, total drug exposure, and weight at last treatment also did not significantly predict latency to conception after final DMPA injection, and explained only 4% of the variance in latency to first conception (Table 4).

Reproductive Output

Mean reproductive output during the reproductive sampling period did not differ between parous-treated subjects (M=1.29±0.99 births) and age-matched controls (M=1.57±0.85 births, t(26)= −0.82, p=0.42; Figure 1). However, mean reproductive output was higher for nulliparous-treated subjects (M=2.06±0.66 births) than for both

age-matched (M=1.29±0.69 births, t(32)=3.32, p=0.002) and experience-matched controls (M=0.88±0.49 births, t(32)=5.93, p<0.001; Figure 1).

Experience-matched controls had a significantly longer mean latency to first birth during the sampling period (M=431.77±114.85 days) than did nulliparous-treated subjects (M=244.77±53.54 days, t(32)=6.08, p<0.001). However, mean latency to first birth did not differ between nulliparous-treated subjects and age-matched controls (M=268.18±223.88 days, t(32)=0.42, p=0.68), or nulliparous-treated subjects and parous-treated subjects (M=234.20±57.07 days, t(25)=0.48, p=0.63).

Pregnancy Outcomes

Parous-treated subjects had a significantly greater proportion of total pregnancies that ended in early abortion (6/24 pregnancies) than did nulliparous-treated subjects (1/36 pregnancies, Fisher's Exact Test (FET), df=1, N=60, p=0.01, Φ=0.34). Because early abortion data were not available for matched controls, pregnancies ending in early abortion were not included in analyses comparing pregnancy outcomes between treated subjects and matched controls.

The most commonly reported negative pregnancy outcomes for treated subjects were perinatal death, maternal abuse/neglect, and stillbirth (Table 5). Treated subjects combined had a greater proportion of total births with negative outcomes (28/52 births) than did age-matched controls (5/52 births, FET, df=1, N=104, p<0.001, Φ=0.48). Looking exclusively at first births within the sampling period, treated subjects still had a significantly greater proportion of first births with negative pregnancy outcomes (15/27 first births) than did age-matched controls (2/27 first births, FET, df=1, N=54, p<0.001,

Φ=0.52). For total births, treated subjects had a significantly higher infant mortality rate (48.1%) than did age-matched controls (9.6%, FET, df=1, N=104, p<0.001, Φ=0.42).

When treated subjects were considered by parity, the proportion of total births with negative outcomes did not differ between parous-treated subjects (8/18 births) and age-matched controls (4/18 births, FET, df=1, N=36, *p*=0.29, Φ=0.24). There was also no difference in infant mortality rate between parous-treated subjects (38.9%) and age-matched controls (22.2%, FET, df=1, N=36, *p*=0.47, Φ=0.18). Conversely, nulliparous-treated subjects had a significantly greater proportion of total births with negative outcomes (20/34 births) than did both age-matched (1/34 births, FET, df=1, N=68, *p*<0.001, Φ=0.61) and experience-matched controls (8/34 births, FET, df=1, N=68, *p*=0.01, Φ=0.36; Figure 2). When only first births within the sampling period were considered, nulliparous-treated subjects still had a significantly greater proportion of first births with negative outcomes (9/17 first births) than both age-matched (0/17 first births, FET, df=1, N=34, p=0.001, Φ=0.60) and experience-matched controls (2/17 first births, FET, df=1, N=34, p=0.03, Φ=0.44). For total births, nulliparous-treated subjects had a significantly higher infant mortality rate (52.9%) than both age-matched (2.9%, FET, df=1, N=68, *p*<0.001, Φ=0.56) and experience-matched controls (23.5%, FET, df=1, N=68, *p*=0.02, Φ=0.30).

The proportion of total births classified as perinatal deaths did not differ between parous-treated subjects (3/18) and age-matched controls (3/18), or between nulliparous-treated subjects (4/34) and age-matched (1/34, FET, df=1, N=68, *p*=0.36, Φ=0.17) or experience-matched controls (7/34, FET, df=1, N=68, *p*=0.51, Φ=0.12).

The proportion of total births classified as cases of maternal abuse/neglect did not differ between parous-treated subjects (2/18 births) and age-matched controls (0/18 births, FET, df=1, N=36, p=0.49, Φ=0.24). Conversely, nulliparous-treated subjects had a significantly greater proportion of total births classified as cases of maternal abuse/neglect (8/34 births) than both age-matched (0/34 births, FET, df=1, N=68, p=0.01, Φ=0.37) and experience-matched controls (0/34 births, FET, df=1, N=68, p=0.01, Φ=0.37; Figure 3). Looking exclusively at first births within the sampling period, the proportion of first births classified as cases of maternal abuse/neglect did not differ between nulliparous subjects (2/17 first births) and age-matched (0/17 first births, FET, df=1, N=34, p=0.49, Φ=0.25) or experience-matched controls (0/17 first births, FET, df=1, N=34, p=0.49, Φ=0.25).

The proportion of total births classified as stillbirths did not differ between parous-treated subjects (2/18 births) and age-matched controls (1/18 births, FET, df=1, N=36, p=1.000, Φ=0.10). Conversely, nulliparous-treated subjects had a significantly greater proportion of total births classified as stillbirths (8/34 births) than both age-matched (0/34 births, FET, df=1, N=68, p=0.01, Φ=0.37) and experience-matched controls (1/34 births, FET, df=1, N=68, p=0.03, Φ=0.30; Figure 3). Looking exclusively at first births within the sampling period, nulliparous-treated subjects still had a significantly greater proportion of first births classified as stillbirths (5/17 first births) than both age-matched (0/17 first births, FET, df=1, N=34, p=0.04, Φ=0.42) and experience-matched controls (0/17 first births, FET, df=1, N=34, p=0.04, Φ=0.42).

When nulliparous-treated subjects were considered by pubertal status, prepubertal nulliparous-treated subjects had a greater proportion of births classified as stillbirths

(6/13 births) than both pubertal nulliparous-treated subjects (2/21 births, FET, df=1, N=34, p=0.03, Φ=0.42) and parous-treated subjects (2/18 births, FET, df=1, N=31, p=0.04, Φ=0.40; Figure 4). During the DMPA treatment period, one parous-treated subject unintentionally received a DMPA injection while pregnant, and the pregnancy subsequently ended in stillbirth.

DISCUSSION

In the present study, chronic DMPA treatment was associated with an increased incidence of negative pregnancy outcomes post-treatment for females placed on treatment as nulliparous adolescents. Nulliparous-treated subjects experienced a higher incidence of stillbirth after DMPA exposure than did control females of the same age, and control females with equivalent reproductive experience. Conversely, we found no evidence of increased incidence of stillbirth post-treatment for females placed on DMPA treatment as parous adults, even though parous-treated subjects were on treatment longer and received more DMPA than did nulliparous-treated subjects. Within the nulliparous-treated subject, the majority of stillbirths were to females who were placed on DMPA treatment prepubertally, indicating that the post-treatment risk of stillbirth following chronic DMPA exposure depended upon the developmental timing of treatment onset. This novel finding raises the question of whether there is a developmental window of vulnerability during which exposure to chronically elevated levels of progestin permanently suppresses future reproductive capacity.

Return of fertility

After DMPA treatment was ended, treated females showed substantial individual variation in latency to return of fertility, as indicted by the resumption of cyclic genital

swelling. However, latency to return of fertility following DMPA treatment cessation was not related to reproductive experience prior to treatment, weight at last treatment, or total DMPA exposure, consistent with findings from studies of human DMPA users (Garza-Florez et al., 1985; Lan et al., 1984; Pardthaisong; 1980). Evidence from studies of human DMPA users suggests that there is substantial individual variation in rate of DMPA metabolism (Fotherby et al., 1980; Schwallie & Assenzo, 1974), thus it is likely that differences in rate of DMPA metabolism and clearance are responsible for the individual variation we found in latency to return of fertility following DMPA treatment cessation.

Reproductive output

In contrast to the relationship between DMPA treatment and negative pregnancy outcomes, rates of *conception* did not differ between treated females and matched controls. These results are consistent with findings from studies of human DMPA users, where conception rates did not differ between previous DMPA users and controls following the resumption of ovulation after treatment cessation (e.g. Schwallie & Assenzo, 1974). The finding that nulliparous-treated subjects exhibited higher reproductive output than their matched controls was initially surprising, however, nulliparous-treated subjects likely exhibited higher reproductive output than their matched controls because they also exhibited higher rates of infant mortality. Female primates experience lactational amenorrhea while nursing their offspring, which lengthens inter-birth intervals (Recabarren et al., 2000; Schallenberger at al., 1981; Stewart, 1988). The death of an infant removes lactational amenorrhea, thus mothers who lose an infant are capable of conceiving again more quickly than mothers who

successfully rear an infant to weaning. Therefore, more frequent infant loss increases reproductive output, but not reproductive success. This is a critical distinction for interpreting the effects of DMPA treatment on future successful reproduction, as the literature emphasizes conception rates for human DMPA users after treatment cessation, while neglecting to further investigate pregnancy outcomes following conception.

Many species of old world primate exhibit a period of adolescent sterility following menarche (Ashley-Montagu, 1939; Resko, et al., 1982; Wallis, 1997). Although nulliparous-treated subjects were adults at sampling period onset, their experience-matched controls were nulliparous adolescents. It is likely that the experience-matched controls went through a period of adolescent sterility following their first ever maximal swelling, which would explain why experience-matched controls had a longer latency to first birth during the sampling period than did nulliparous-treated subjects. This delay in first birth for experience-matched controls provides an additional explanation for the comparably higher reproductive output of nulliparous-treated subjects. Interestingly, latency to first birth did not differ between nulliparous-treated subjects and their age-matched controls, indicating that nulliparous-treated subjects did not experience a period of adolescent sterility after the cessation of DMPA treatment, even though they began receiving DMPA either before or directly after their first ever maximal swelling. Therefore, the nulliparous-treated subjects passed through their window of adolescent sterility while receiving DMPA, although hormonal fluctuation and ovulation were being suppressed. These results suggest that adolescent sterility may be regulated chronologically and not as a result of the duration of ovarian function. However, this does not rule that adolescent endocrine experience has no effect on future

reproductive potential, as indicated by our finding of increased negative pregnancy outcomes for nulliparous-treated subjects.

The delay in first birth for experience-matched controls as compared to nulliparous-treated subjects may also represent behavioral differences between the two groups. Again, the experience-matched controls were much younger than the nulliparous-treated subjects at sampling period onset, and therefore they may have shown less willingness to mate with adult males in the group, or conversely, adult males may have shown less willingness to mate with them. For many species of old world primates, adult males exhibit a preference for mating with older, parous females as opposed to younger, nulliparous females, although such a preference in sooty mangabeys has not been directly investigated (for review see Anderson, 1986).

Pregnancy Outcomes

Advanced maternal age increases the risk of spontaneous early abortion in both human and nonhuman primates (Heffner, 2004; Schlabritz-Loutsevitch, et al., 2008; Schramm & Bavister, 1999). Therefore, it is likely that parous-treated subjects exhibited a higher incidence of early abortion than nulliparous-treated subjects because they were significantly older than the nulliparous-treated subjects (see Table 2). The early abortion rate of 25% for parous-treated subjects is comparable to rates of spontaneous abortion reported for women of advanced maternal age (e.g. Heffner, 2004). However, lack of early abortion data for matched controls meant that we could not determine whether the rate of early abortion we found for parous-treated subjects was greater than would be expected based solely on maternal age.

Likelihood of perinatal death did not differ between treated subjects and their matched controls, indicating that DMPA treatment had no effect on this endpoint. Conversely, nulliparous-treated subjects were significantly more likely to engage in maternal abuse and/or neglect towards their infants than both age-matched and experience-matched controls, indicating that this increased incidence of poor maternal care did not reflect a lack of reproductive experience. Although likely not a direct consequence of DMPA exposure, the high incidence of poor maternal care in nulliparous-treated subjects may represent a lack of pre-adult experience with infants. All adult females in the social group containing the treated subjects were placed on DMPA treatment while the nulliparous-treated subjects were still juveniles, and consequently there were no infants in the group while nulliparous-treated subjects were pre-adults. Therefore, the nulliparous-treated subjects had no opportunity to interact with infants until they gave birth to their own. Evidence from several species of nonhuman primates suggests that pre-adult experience with infants leads to greater infant survival in adulthood (for review see Pryce, 1996). For nulliparous-treated subjects, a lack of pre-adult experience with infants may have led to an increased rate of poor maternal care following DMPA treatment cessation.

Stillbirths

Maternal age and/or lack of reproductive experience were not responsible for the increased risk of stillbirth in nulliparous-treated subjects, given that nulliparous-treated females had an increased incidence of stillbirth as compared to both age-matched and experience-matched controls. Furthermore, average group size was greater for age-matched and experience-matched controls than for nulliparous-treated subjects,

suggesting that group density was not responsible for the increased incidence of stillbirth in nulliparous-treated females. When only first births within the sampling period were considered, we still found an increased incidence of stillbirth for nulliparous-treated females, indicating that this finding was not a product of the fact that certain treated females contributed more births towards total births during the sampling period than others. Taken together these findings strongly suggest that DMPA exposure increased likelihood of stillbirth in females placed on DMPA treatment as nulliparous adolescents.

The increased risk of stillbirth for females placed on DMPA during early adolescence was associated with an increased incidence of labor complications post-treatment. Five out of the eight cases of stillbirth to nulliparous-treated subjects were observed with accompanying prolonged and/or difficult labor (dystocia), which was ultimately determined to be the cause of infant death. For the other three cases of stillbirth to nulliparous-treated subjects, the females were not directly observed during labor, however necropsy of the stillborn infants did not reveal any infant abnormalities that could explain the infants' deaths. Thus it is likely that dystocia was the cause of all stillbirths to nulliparous-treated subjects.

In humans, dystocia is associated with insufficient uterine contraction strength (Seitchik, 1987), and progestins have long been known to suppress uterine contractility (Csapo & Goodall, 1954; Putnam et al., 1991; Ruddock et al., 2008). Women who go into preterm labor are often treated with progestins to suppress uterine contractions and delay labor (Seitchik, 1987). In primates, the uterus continues to develop postnatally until puberty, and this postnatal uterine development is sensitive to levels of progestins (Yin & Ma, 2005). Evidence from studies of animals suggests that exposure to elevated levels of

progestin during species-specific sensitive periods of uterine development can permanently alter the structure of the adult uterus (for review see Gray et al., 2001). Our results point to a possible relationship between prepubertal exposure to the synthetic progestin DMPA and an increased likelihood of dystocia and stillbirth years after DMPA treatment has ended. It is possible that exposure to chronically elevated levels of progestin during adolescence, a critical period of uterine development, permanently reduces uterine contractility and increases the likelihood of dystocia and stillbirth for females placed on DMPA treatment during early adolescence.

The findings of the present study indicate that DMPA exposure during early adolescence may permanently decrease post-treatment reproductive capacity. There are currently no age restrictions on the use of DMPA in humans, and DMPA is frequently administered to adolescent girls (Cromer et al., 1998). Given that female sooty mangabeys share many aspects of reproductive physiology and endocrinology with women, the results of the present study raise questions about the safety of administering DMPA to adolescent girls.

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TABLE 1 – Grouping of DMPA-treated subjects

Group	Sub-Group	N	At Time of First DMPA Injection
Parous Treated		14	Adult females with at least one previous birth
Nulliparous Treated		17	Adolescent females who had never given birth
	Prepubertal	7	Adolescent females who had never achieved maximal perineal swelling
	Pubertal	10	Adolescent females who had achieved one maximal perineal swelling

DMPA = depot medroxypogesterone acetate

TABLE 2 – Descriptives for parous versus nulliparous-treated subjects

	N	Age at		Total	
		First Treatment (yr)	Last Treatment (yr)	Time on Treatment (yr)	Drug Exposure (mg)
Parous Treated	14	6.72±0.35	14.82±0.39	8.10±0.06	5090.36±148.31
Nulliparous Treated	17	3.25±0.07**	9.08±0.29**	5.83±0.26**	4503.53±172.77*

mean ± SE

* Nulliparous treated significantly differed from parous treated at the level of $p < 0.05$

** Nulliparous treated significantly differed from parous treated at the level of $p < 0.001$

TABLE 3 – Definitions for classification of pregnancy outcomes

CATEGORY	TYPE	DEFINITION
Positive Outcomes	Live Birth	Full-term infant delivered vaginally and surviving more than 28 postnatal days
	Planned C-Section	Full-term infant delivered via planned cesarean section surviving more than 28 postnatal days
Negative Outcomes	Early Abortion	Spontaneous miscarriage of fetus prior to viability
	Stillbirth	Full-term infant born deceased
	Perinatal Death	Infant died within 28 postnatal days for health reasons other than maternal abuse
	Maternal Abuse/Neglect	Full-term infant abused or neglected by dam, died or was removed from dam within 28 postnatal days
	Premature	Pre-term infant, survived more than 28 postnatal days

TABLE 4 – Summary of multiple linear regression models predicting a) latency to resumption of ovulation and b) latency to conception after final DMPA injection

(a)

	B	SE B	β	*p*
Constant	217.33	97.98		0.04
Parity at first treatment	6.47	20.75	0.07	0.76
Total drug exposure	< 0.01	0.02	0.02	0.93
Weight at last treatment	-5.12	7.07	-0.14	0.48

R^2=0.02, F=0.21

(b)

	B	SE B	β	*p*
Constant	148.79	162.55		0.37
Parity at first treatment	-2.42	35.06	-0.02	0.95
Total drug exposure	0.02	0.03	0.18	0.42
Weight at last treatment	2.32	11.70	0.04	0.84

R^2=0.04, F=0.37

TABLE 5 – Occurrence of pregnancy outcomes during sampling period by group

TYPE	Parous Treated	AM Controls	Nulliparous Treated	AM Controls	EM Controls
Live Birth	10	14	12	32	25
Planned C-section	0	0	1	0	0
Stillbirth	2	1	8	0	1
Perinatal Death	3	3	4	1	7
Maternal Abuse/Neglect	2	0	8	0	0
Premature	1	0	0	0	0
Total Births	18	18	33	33	33

FIGURE CAPTIONS

FIGURE 1– Mean number of births within the sampling period by group. Number of births was examined over the same number of days of reproductive opportunity for treated subjects and their matched controls. Parous-treated subjects did not differ from age-matched (AM) controls. Nulliparous-treated subjects had significantly greater reproductive output than did age-matched (AM) or experience-matched (EM) controls. Error bars represent 1 standard error from the mean.

* Significant difference at level of <0.05

** Significant difference at level of <0.001

FIGURE 2– Percentage of total births during the reproductive sampling period categorized as negative pregnancy outcomes by group. Parous-treated subjects did not differ from age-matched (AM) controls. Nulliparous-treated subjects had a significantly higher incidence of negative pregnancy outcomes than did age-matched (AM) or experience-matched (EM) controls.

* Significant difference at level of <0.05

** Significant difference at level of <0.001

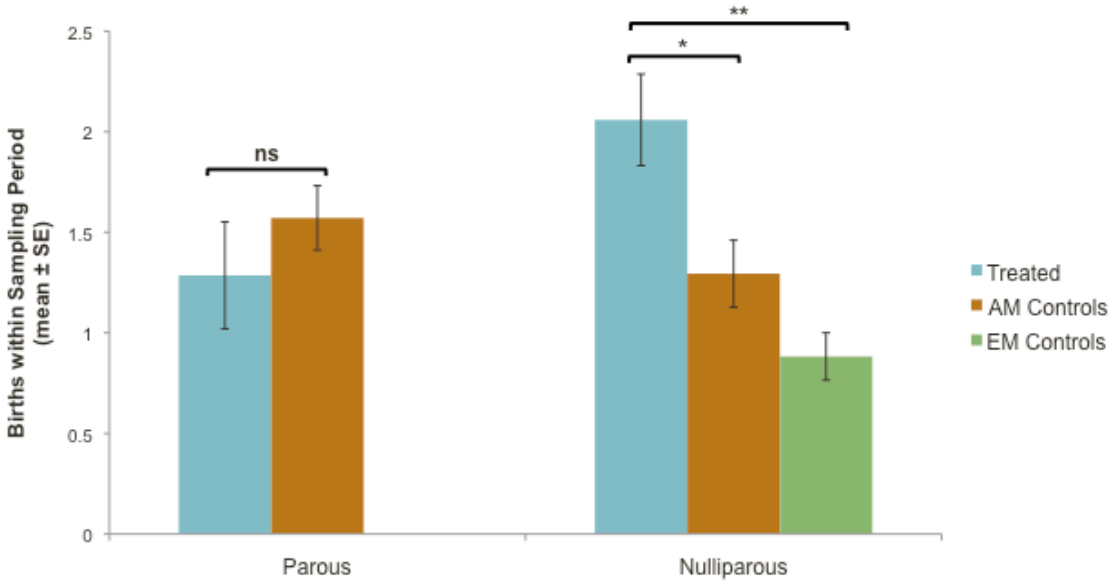
FIGURE 3– Percentage of total births during the reproductive sampling period classified as stillbirths or cases of maternal abuse/neglect by group. Parous-treated subjects did not differ from age-matched (AM) controls. Nulliparous-treated subjects had a significantly higher incidence of both stillbirth and maternal abuse/neglect than did age-matched (AM) or experience-matched (EM) controls.

* Significant difference at level of <0.05

FIGURE 4– Percentage of births during the reproductive sampling period classified as stillbirths for treated subject by pubertal status. Prepubertal nulliparous-treated subjects had a significantly higher incidence of stillbirth than did parous-treated subjects or pubertal nulliparous-treated subjects.

* Significant difference at level of <0.05

FIGURE 1 –


Births within Sampling Period (mean ± SE)
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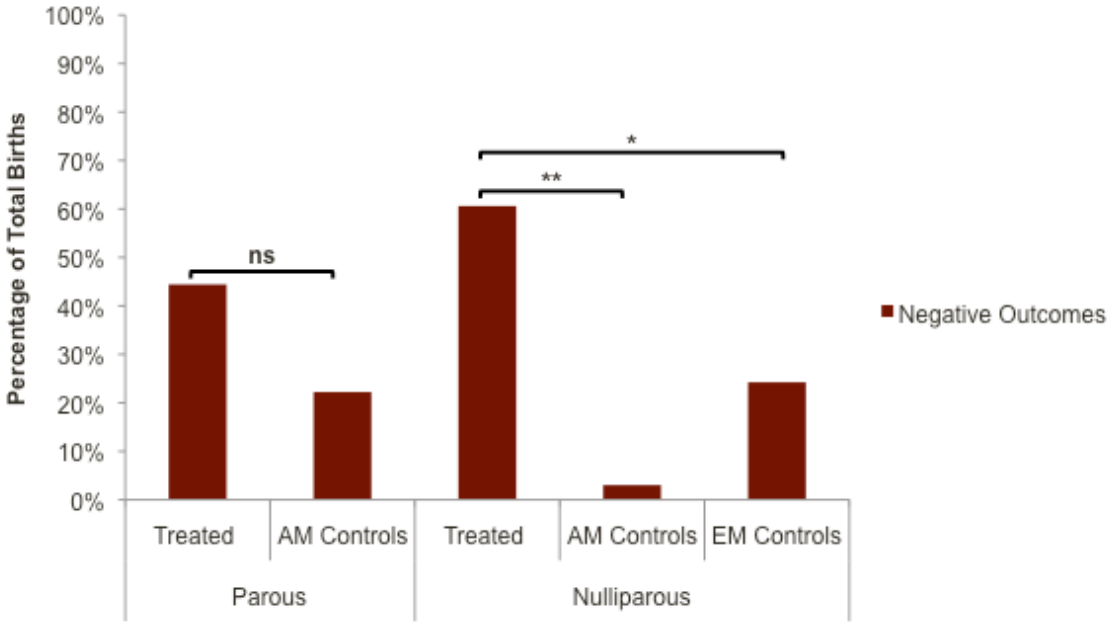
**
Parous
Nulliparous
Treated
AM Controls
EM Controls

FIGURE 2 –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Percentage of Total Births
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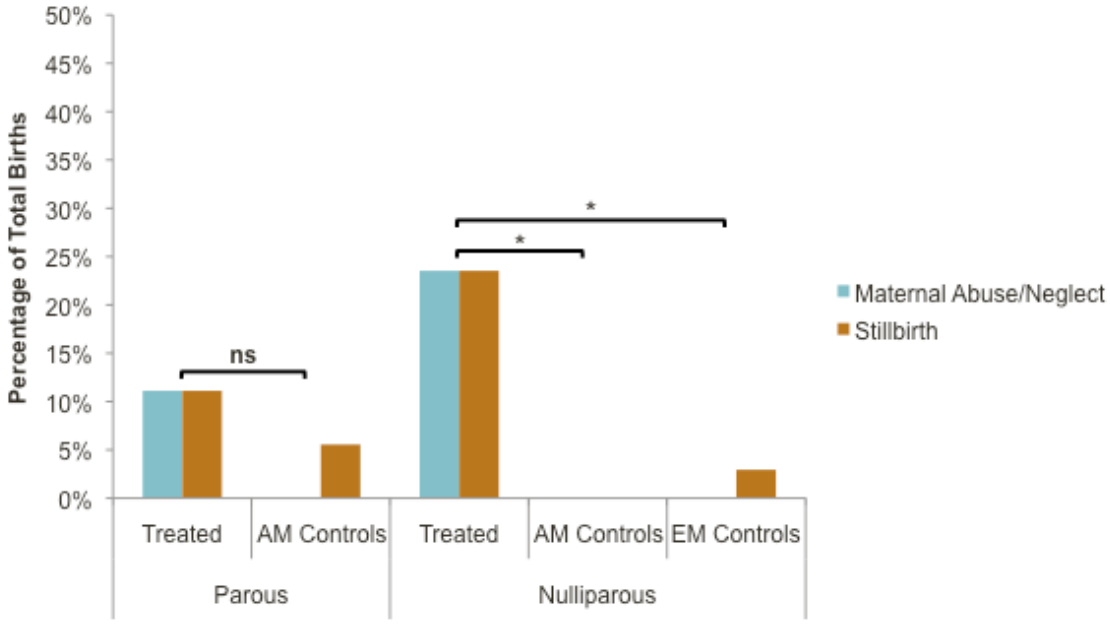
Treated
AM Controls
Treated
AM Controls
EM Controls
Parous
Nulliparous
Negative Outcomes

FIGURE 3 –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Maternal Abuse/Neglect
Stillbirth
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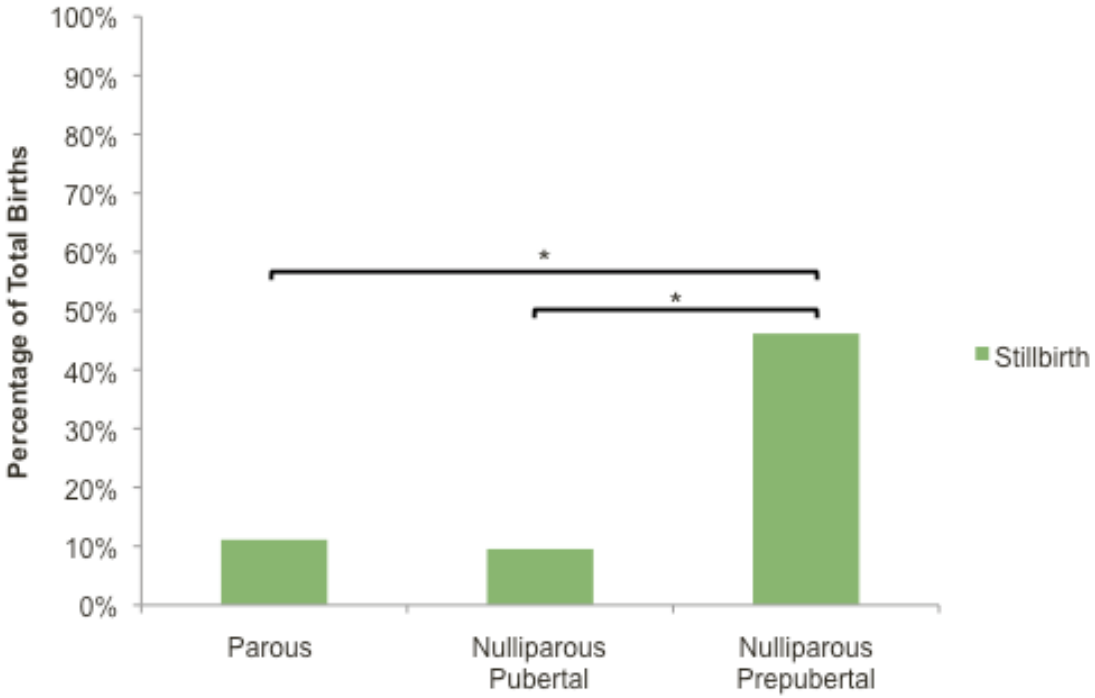
Treated
AM Controls
EM Controls
Parous
Nulliparous

FIGURE 4 –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Stillbirth
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Nulliparous Pubertal
Nulliparous Prepubertal