Early adolescent Depo-Provera exposure increases stillbirths in adult sooty mangabeys

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Abstract

The 3-month injectable contraceptive medroxyprogesterone acetate (MPA; Depo-Provera) is a synthetic progestin that protects against pregnancy by suppressing ovulation. Studies have focused on the resumption of ovulation after MPA-treatment cessation but neglected potential long-term effects of MPA exposure on future successful reproduction. MPA is frequently administered to adolescent girls; however, long-term fertility effects of adolescent MPA exposure have not been explored. We investigated fertility after extended MPA exposure in a species of old world primate, the sooty mangabey (*Cercocebus atys*). Female sooty mangabeys ($n=31$) received chronic MPA-treatment for 4–8 years. At MPA-treatment onset, subjects were either parous adults ($n=14$) or nulliparous adolescents ($n=17$), with adolescent-treated subjects being further divided into those who had reached first ovulation ($n=10$) and those who had not ($n=7$). After MPA-treatment cessation, adolescent-treated females had a significantly higher incidence of stillbirth than did age-matched and parity-matched controls, whereas adult-treated females did not differ from their matched controls. Females placed on MPA-treatment prior to first ovulation had a significantly higher incidence of stillbirth post-treatment than did females placed on MPA-treatment after first ovulation. Diabetic females had an increased incidence of stillbirth as compared to nondiabetic females; however, when controlling for diabetes, MPA exposure prior to first ovulation was still a significant positive predictor of stillbirth. These findings suggest that the post-treatment fertility effects of chronic MPA exposure vary with the developmental timing of treatment onset and raise concern about the use of MPA as a contraceptive for adolescent girls.

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Introduction

The 3-month injectable contraceptive medroxyprogesterone acetate (MPA; Depo-Provera) is a synthetic progestin that protects against pregnancy by acting at the level of the pituitary and hypothalamus to suppress ovulation (Rivera *et al.* 1999). MPA is currently approved for human use in over 90 countries worldwide (Khoiny 1996), and since its approval by the Food and Drug Administration (FDA) in 1992, over 11 million women in the United States have used MPA (Mosher & Jones 2010).

Return of fertility after MPA-treatment cessation is of concern to both MPA users and prescribing physicians; however, studies examining fertility after MPA exposure have largely focused on resumption of ovulation. Some evidence suggests that women previously exposed to MPA may be at an increased risk of adverse pregnancy outcomes. In a single cohort of women living on the island of Jamaica, Greenwood and McCaw-Binns (1994) and Greenwood *et al.* (1994) reported that previous Depo-Provera use (modeled with maternal age) was a significant positive predictor of stillbirth, even when controlling for factors such as drug and alcohol use and

maternal nutrition; however, such associations can be difficult to interpret in women given an unavoidable lack of environmental controls.

Many clinicians recommend prescribing MPA instead of oral contraceptives specifically for adolescent patients to avoid compliance issues associated with a daily pill regimen (Cromer *et al.* 1998, Khoiny 1996, Tolaymat & Kaunitz 2007). Between 2006 and 2008, over 270 000 adolescent girls (ages 15–19) in the United States alone were using MPA (Mosher & Jones 2010). Despite the large number of adolescent MPA users, the possibility of differential long-term fertility effects of MPA exposure during adolescence has not been explored.

Captive nonhuman primates provide an opportunity to investigate the long-term fertility effects of synthetic progestin exposure in a controlled environment. No studies have explicitly investigated the effects of MPA exposure on future infant survival in nonhuman primates; however, two studies examined the fertility effects of exposure to a similar synthetic progestin, melengestrol acetate (MGA). Unlike MPA, MGA is administered via subdermal implant; however, both

contraceptives protect against pregnancy via the same mechanism. Wood *et al.* (2001) reported that female golden lion tamarins (*Leontopithecus rosalia*) exposed to MGA had a significantly higher risk of stillbirth after MGA-treatment cessation than did nonexposed controls. A portion of their sample, however, was comprised of females whose MGA capsules had been exhausted but not surgically removed. These “empty” MGA capsules 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 fertility after MGA exposure in golden-headed lion tamarins (*Leontopithecus chrysomelas*) but limited their analysis to females whose MGA capsules had been surgically removed. The authors reported that females had a significantly higher risk of stillbirth after MGA-treatment cessation as compared to before they were exposed to MGA; however, these females were also several years older after MGA treatment as compared to before, and the authors did not control for maternal age. Nonetheless, both of these studies suggest the need for further investigation into the long-term effects of synthetic progestin exposure on future infant survival in nonhuman primates.

The present study examined the return of fertility, reproductive output, and pregnancy outcomes after extended MPA exposure in a species of the old-world primate, the sooty mangabey (*Cercocebus atys*). Sooty mangabeys are menstrual primates with an average menstrual cycle length within the normal human range (Hadidian & Bernstein 1979) and patterns of hormonal fluctuation across the menstrual cycle very similar to those in women (Aidara *et al.* 1981). Sooty mangabeys therefore represent an appropriate animal model for the study of processes relevant to human reproductive endocrinology. We report here that the fertility consequences of extended MPA exposure in sooty mangabeys varied with the developmental timing of MPA-treatment onset.

Materials and methods

Data in this study were obtained from historical clinical records of the Yerkes National Primate Research Center. All clinical procedures described in this study were approved by the Emory University Institutional Animal Care and Use Committee under the Maintenance of the Yerkes National Primate Center Animal Colony Protocol (#2003042).

Treated subjects

MPA-treated subjects

Treated subjects were female sooty mangabeys (*Cercocebus atys*; $n=31$) living in one large multi-male/multi-female social group at the Yerkes National Primate

Research Center (YNPRC) Field Station in Lawrenceville, GA, USA, housed in a large outdoor enclosure ($\sim 230\text{ m}^2$) with attached indoor quarters ($\sim 16.5\text{ m}^2$). All treated subjects were placed on MPA-treatment for population management, and not for health concerns. At the time of MPA-treatment onset, treated subjects were either parous adults ($n=14$; 1–3 births) or nulliparous adolescents ($n=17$), with adolescent-treated subjects being further defined as ovulatory ($n=10$) or pre-ovulatory ($n=7$). Following an established protocol for estimating ovulation in sooty mangabeys, the last day of maximal perineal swelling during a given menstrual cycle was considered the day of ovulation (Aidara *et al.* 1981). Adolescent-treated subjects were defined as pre-ovulatory if they had never achieved maximal perineal swelling when MPA-treatment began. Menstruation data were not available, and the pre-ovulatory adolescent-treated subjects may or may not have reached menarche at the time of MPA-treatment onset. The reproduction of each treated subject was examined via clinical records after MPA-treatment cessation ($M=1.97 \pm 0.64$ years).

MPA-treatment administration

Treated subjects received original brand Depo-Provera and/or generic depot medroxyprogesterone acetate (obtained from various suppliers) via i.m. injection every 1–3 months for durations lasting 4–8 years. Adult-treated subjects were, on average, older at the time of their first MPA injection, on MPA-treatment longer, and received a greater total amount of MPA over the course of the treatment period than did adolescent-treated subjects (Table 1).

MPA injection dosage varied between 15 and 300 mg and increased on a calendar method that was not based on weight throughout the treatment period in an attempt to achieve a dosage that would continuously suppress ovulation in all treated females. Four adult-treated subjects became pregnant during the treatment period. One of these females unintentionally received a MPA injection while pregnant, and that pregnancy subsequently ended in a stillbirth. The other three females received no further MPA injections during gestation and/or lactation, and MPA injections were resumed after their infants were weaned. One adolescent-treated subject included in this study was the product of one of these unintended pregnancies during the treatment period. While this subject was born of a mother exposed to MPA, it is unlikely that this female was exposed to MPA prenatally or while nursing, as her mother received no MPA injections during gestation and/or lactation.

Controls

All adult female sooty mangabeys in the YNPRC Field Station breeding colony were placed on MPA-treatment starting in October 2001; therefore, there were no

Table 1 Comparison of measures between adult- and adolescent-treated subjects.

Treatment group	n	Age at		Total	
		First treatment (year)	Last treatment (year)	Time on treatment (year)	Drug exposure (mg)
Adult-treated	14	6.72 ± 0.35	14.82 ± 0.39	8.10 ± 0.06	5090.36 ± 148.31
Adolescent-treated	17	3.25 ± 0.07 ^a	9.08 ± 0.29 ^a	5.83 ± 0.26 ^a	4503.53 ± 172.77 ^b

Mean ± S.E.M.

^aAdolescent-treated significantly differed from adult-treated at the level of $P < 0.001$. ^bAdolescent-treated significantly differed from adult-treated at the level of $P < 0.05$.

untreated females available to act as contemporary controls. Control females were randomly selected from the colony's historical records, and their reproduction was retrospectively examined during a time period prior to colony-wide MPA-treatment onset. As this was a retrospective observational study, the historical controls did not receive vehicle injections. No female acted as both a treated subject and a control.

Age-matched controls

To control for effects of maternal age on successful reproduction, an age-matched control was selected from historical records for each treated subject ($n = 31$). The reproduction of each age-matched control was retrospectively followed from the day when the control would have been the same age as their matched treated subject (e.g., if a treated subject was 3500 days old on the day of their first ovulation after MPA-treatment cessation, then the reproduction of their age-matched control was retrospectively followed from the historical day when the control would have also been 3500 days old). All age-matched controls had given birth at least once.

Parity-matched controls

The adolescent-treated subjects had all reached adulthood by the end of the MPA-treatment period but had still never given birth (unlike their parous age-matched controls). To control for effects of reproductive experience on successful reproduction, a parity-matched control was selected from historical records for each adolescent-treated subject ($n = 17$). The reproduction of each parity-matched control was retrospectively followed from the day of their first ovulation (i.e., all parity-matched controls were nulliparous adolescents).

Following reproduction

Return of fertility

Latency to return of fertility after MPA-treatment cessation was measured by both latency to resumption of ovulation (defined as the number of days from final MPA injection to first subsequent ovulation) and latency

to first birth (defined as the number of days from final MPA injection to first subsequent birth).

Reproductive output

Reproductive output was defined as the total number of births across total number of days of reproductive opportunity during the study period. 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 fertile 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. The reproductive output of each age-matched and parity-matched control was examined over the same number of days of reproductive opportunity as their matched treated subject.

Pregnancy outcomes

Each birth during the study period was categorized as one of the following pregnancy outcomes: live birth, planned C-section, stillbirth, neonatal death, maternal abuse/neglect, or premature birth (based on the definitions in Table 2). The percentage of total births during the study period categorized as each type of pregnancy outcome was calculated. All treated subjects had 0–3 births during the study period, and thus some treated subjects contributed more births toward the total count of births than did others. To account for the overrepresentation of certain treated subjects, each age-matched and parity-matched control was followed for the same number of births as was their matched treated subject. To further account for the discrepancy in births contributed by each treated subject, all pregnancy outcome analyses were additionally performed for first births only during the study period.

Perineal swelling data collection

Trained observers visually inspected all female sooty mangabeys 5 days per week and rated perineal swelling size on a scale from 0 to 4, with 4 indicating maximal swelling. Two observers collected all perineal swelling data used in this study with an inter-observer reliability of 85% agreement on swelling size. The same perineal

Table 2 Definitions for categorization of pregnancy outcomes.

Category	Type	Definition
Positive outcomes	Live birth	Full-term infant delivered vaginally; survived more than 28 postnatal days
	Planned C-section	Full-term infant delivered via planned cesarean section; survived more than 28 postnatal days
Negative outcomes	Stillbirth	Full-term infant born deceased (without having taken a breath)
	Neonatal 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 birth	Pre-term infant; survived more than 28 postnatal days

swelling data collection protocol was used for all subjects, including historical controls.

Diabetes

Of the 31 treated subjects, 5 were determined to be diabetic during the study period. Diabetes has been shown to impact pregnancy outcomes in women (Lauenborg *et al.* 2003, Wood *et al.* 2003, Mathiesen *et al.* 2011), and we therefore examined the impact of diabetes on pregnancy outcomes in the treated subjects. Breeding female sooty mangabeys were determined to be diabetic if they met two of the four criteria: persistent hyperglycemia, elevated fructosamine, presence of glucosuria, and/or weight loss. Hyperglycemia was defined by a fasting blood glucose level greater than 175 mg/dl (Jones *et al.* 2014). Animals with fructosamine levels greater than 300 µmol/l were considered diabetic (Jones *et al.* 2014). The incidence of diabetes did not differ between the adult- (14%) and adolescent-treated subjects (18%; Fisher's exact test (FET), degrees of freedom (df)=1, n =31, P =1.00, Φ =0.05). Diabetic mangabeys were not treated for their diabetes. Diabetes treatment requires daily monitoring of blood glucose levels and adjustments of medication dosage. These animals lived outside in large social groups, preventing daily accesses for blood collection. Furthermore, a major risk of treatment with diabetic drugs includes acute hypoglycemic episodes. In our experience, sooty mangabeys can live for years in a diabetic state without common diabetic sequelae, such as cardiovascular disease, nephropathy, and retinopathy. Thus, the risks of treatment for diabetes outweigh the benefits in these animals. Diabetes data were not available for historical controls.

Statistical analysis

All analyses used SPSS software (version 22.0.0, IBM Corp., Somers, NY, USA). All tests were two-tailed and considered significant with a P value less than 0.05. Group mean differences were assessed with the independent samples t -test, using Cohen's d as a measure of effect size (Cohen 1977). Multiple linear regression was used to examine the relationship between parity at first treatment, total drug exposure, weight at last treatment, and latency to return of fertility after MPA-treatment cessation.

Differences between groups in the proportion of total births and first births with negative outcomes during the study period were analyzed with the Fisher's exact test (FET), using the phi coefficient as a measure of effect size (Cohen 1977). A binary logistic regression model (with pregnancies nested within dams) was used to examine the relationship between total drug exposure, ovulatory status at treatment onset, diabetes, and stillbirth. A one-way analysis of variance (ANOVA) was used to examine the relationship between birth number after final MPA injection (first, second, or third birth) and stillbirth. When attempting to balance the risk of type I versus type II 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 II error was more serious. We therefore maintained an alpha level of 0.05 but did not systematically correct for multiple comparisons (Bender & Lange 2001).

Results

Return of fertility

One adult-treated female mangabey failed to resume ovulation within the study period after her final MPA injection (937 days) and was excluded from the study. Latency to resumption of ovulation after the final MPA injection varied between 106 and 317 days, and mean latency to resumption of ovulation did not differ between adult-treated (M =185.86, $s.d.$ =55.80 days) and adolescent-treated subjects (M =191.35, $s.d.$ =45.34 days, $t(29)$ =0.30, P =0.76, Cohen's d =0.11). Four adult-treated subjects failed to give birth again within the study period after their final MPA injections and were excluded from analysis of latency to first birth. Latency to first birth after the final MPA injection varied between 328 and 669 days, and mean latency to first birth also did not differ between adult-treated (M =419.80, $s.d.$ =97.76 days) and adolescent-treated subjects (M =436.12, $s.d.$ =54.43 days, $t(25)$ =0.56, P =0.58, Cohen's d =0.21).

Parity at first treatment, total drug exposure, and weight at last treatment did not significantly predict latency to resumption of ovulation or latency to first birth after final MPA injection (Table 3).

Table 3 Summary of multiple linear regression model predicting (a) latency to resumption of ovulation after final MPA injection and (b) latency to first birth after final MPA injection.

Predictors	B	S.E.M.	β	P
Latency to resumption of ovulation after final MPA injection ^a				
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
Latency to first birth after final MPA injection ^b				
Constant	410.94	150.22		0.12
Parity at first treatment	27.22	33.06	0.19	0.42
Total drug exposure	0.01	0.02	0.13	0.56
Weight at last treatment	-10.13	11.04	-0.19	0.37

^a $R^2=0.02$, $F=0.21$. ^b $R^2=0.05$, $F=0.43$.

Reproductive output

Mean reproductive output during the study period did not differ between adult-treated subjects ($M=1.29$, $S.D.=0.99$ births) and age-matched controls ($M=1.57$, $S.D.=0.85$ births; $t(26)=-0.82$, $P=0.42$, Cohen's $d=0.31$). Adolescent-treated subjects had a significantly higher mean reproductive output ($M=2.06$, $S.D.=0.66$ births) than did age-matched ($M=1.29$, $S.D.=0.69$ births; $t(32)=3.32$, $P=0.002$, Cohen's $d=1.14$) and parity-matched controls ($M=0.88$, $S.D.=0.49$ births; $t(32)=5.93$, $P<0.001$, Cohen's $d=2.03$).

Pregnancy outcomes

The most commonly observed negative pregnancy outcomes during the study period were neonatal death, maternal abuse/neglect, and stillbirth (Table 4). The incidence of neonatal death did not significantly differ between adult-treated subjects (17%) and age-matched controls (17%; FET, $df=1$, $n=36$, $P=1.00$, $\Phi=0.00$) or between adolescent-treated subjects (12%) and age-matched (3%; FET, $df=1$, $n=68$, $P=0.36$, $\Phi=0.17$) or parity-matched controls (21%; FET, $df=1$, $n=68$, $P=0.51$, $\Phi=0.12$; Table 4).

The incidence of maternal abuse/neglect did not significantly differ between adult-treated subjects (11%) and their age-matched controls (0%; FET, $df=1$, $n=36$, $P=0.49$, $\Phi=0.24$). However, adolescent-treated

subjects had a significantly higher incidence of maternal abuse/neglect (24%) than did both their age-matched (0%; FET, $df=1$, $n=68$, $P=0.01$, $\Phi=0.37$) and parity-matched controls (0%; FET, $df=1$, $n=68$, $P=0.01$, $\Phi=0.37$; Table 4). Looking exclusively at first births within the study period, the incidence of maternal abuse/neglect did not significantly differ between adolescent-treated subjects (12%) and their age-matched (0%; FET, $df=1$, $n=34$, $P=0.49$, $\Phi=0.25$) or parity-matched controls (0%; FET, $df=1$, $n=34$, $P=0.49$, $\Phi=0.25$).

The incidence of stillbirth did not significantly differ between adult-treated subjects (11%) and age-matched controls (6%; FET, $df=1$, $n=36$, $P=1.00$, $\Phi=0.10$; Fig. 1). Conversely, adolescent-treated subjects had a significantly higher incidence of stillbirth (24%) than did both age-matched (0%; FET, $df=1$, $n=68$, $P=0.01$, $\Phi=0.37$) and parity-matched controls (3%; FET, $df=1$, $n=68$, $P=0.03$, $\Phi=0.30$; Table 4; Fig. 1). Looking exclusively at first births within the study period, adolescent-treated subjects still had a significantly higher incidence of stillbirth (29%) than did both age-matched (0%; FET, $df=1$, $n=34$, $P=0.04$, $\Phi=0.42$) and parity-matched controls (0%; FET, $df=1$, $n=34$, $P=0.04$, $\Phi=0.42$).

Looking exclusively within treated subjects, females placed on MPA treatment prior to first ovulation had a significantly higher incidence of stillbirth (46%) than did females placed on MPA treatment after first ovulation (10%, FET, $df=1$, $n=52$, $P=0.01$, $\Phi=0.39$; Fig. 2). When controlling for dam, diabetes, and total drug exposure, MPA exposure prior to first ovulation remained a significant positive predictor of stillbirth after the MPA-treatment cessation (Table 5). When controlling for dam, ovulatory status at MPA-treatment onset, and total drug exposure, diabetes was also a significant positive predictor of stillbirth (Table 5). When controlling for dam, diabetes, and ovulatory status at MPA-treatment onset, total drug exposure was not a significant predictor of stillbirth following MPA-treatment cessation (Table 5). There was no effect of birth number after the final MPA injection (first, second, or third birth) on stillbirth ($F(2,57)=1.00$, $P=0.37$). The adolescent-treated subject born to a dam with previous MPA-exposure experienced no negative pregnancy outcomes during the study period.

Table 4 Comparison of pregnancy outcomes during sampling period by treatment and control groups.

Outcome	Adult-treated	Age controls	Adolescent-treated	Age controls	Parity controls
Live birth	10 (56)	14 (78)	13 (38) ^{a,b}	33 (97)	26 (76)
Planned C-section	0	0	1 (3)	0	0
Stillbirth	2 (11)	1 (6)	8 (24) ^{c,b}	0	1 (3)
Neonatal death	3 (17)	3 (17)	4 (12)	1 (3)	7 (21)
Maternal abuse/neglect	2 (11)	0	8 (24) ^{c,b}	0	0
Premature birth	1 (6)	0	0	0	0
Total births	18	18	34	34	34

Number of births (% of total births).

^aAdolescent-treated significantly differed from age controls at the level of $P<0.001$. ^bAdolescent-treated significantly differed from parity controls at the level of $P<0.05$. ^cAdolescent-treated significantly differed from age controls at the level of $P<0.05$.

Figure 1 Percentage of total births categorized as stillbirths by treatment group. The incidence of stillbirth was examined over the same number of births for treated subjects and their matched controls. Adult-treated subjects did not differ from their age-matched controls. Adolescent-treated subjects had a significantly higher incidence of stillbirth after MPA-treatment cessation than did their age-matched or parity-matched controls. * $P < 0.05$; NS, not significant.

Discussion

In the present study, all but one MPA-treated female resumed ovulating within the study period following their final MPA injections. There was no difference in latency to return of fertility after the final MPA injection for females placed on MPA treatment as nulliparous adolescents vs parous adults; however, the post-treatment incidence of stillbirth varied significantly with the developmental timing of treatment onset.

Stillbirth

Females placed on MPA treatment as nulliparous adolescents had an increased incidence of stillbirth post-treatment as compared to both untreated females of the same age and untreated females with equivalent reproductive experience. Conversely, we found no evidence of increased stillbirths post-treatment for females placed on MPA treatment as parous adults, even though the adult-treated females were on treatment longer and were exposed to more MPA over the treatment period than were the adolescent-treated females. The incidence of diabetes did not differ between the adult- and adolescent-treated females, suggesting that diabetes was not the cause of the increased incidence of stillbirth for the adolescent-treated females. When considering only first births, adolescent-treated females still had an increased incidence of stillbirth as compared to their matched controls, indicating that this finding was not related to the fact that some treated females contributed more births toward the total count of births during the study period than did others. Several adolescent-treated females experienced repeated stillbirths, and stillbirths even three pregnancies out from (and more than 3 years after) their final MPA injections,

indicating that stillbirth was not the result of lingering MPA (Schwallie & Assenzo 1974, Fotherby *et al.* 1980).

Within the treated subjects, we found that females exposed to MPA prior to first ovulation had a significantly higher incidence of stillbirth post-treatment as compared to females placed on MPA treatment at any point after first ovulation (as nulliparous ovulatory adolescents or parous adults). In total, females placed on MPA treatment prior to first ovulation had a stillbirth rate of 46%, as compared to only 10% for females placed on MPA treatment after first ovulation (Fig. 2). We also found that, within treated subjects, diabetic females had an increased incidence of stillbirth as compared to nondiabetic females. This finding is consistent with findings from studies of pregnancy outcomes in diabetic women, in which diabetes has been shown to be a risk factor for stillbirth (Lauenborg *et al.* 2003, Wood *et al.* 2003, Mathiesen *et al.* 2011). Importantly, even when controlling for diabetes, exposure to MPA prior to first ovulation was still a positive predictor of stillbirth. These findings indicate that diabetes and exposure to MPA prior to first ovulation are independent risk factors for stillbirth.

Taken together, these findings strongly suggest that MPA exposure increased the likelihood of stillbirth post-treatment only for females placed on MPA-treatment in early adolescence and that MPA exposure most severely affected females placed on MPA-treatment prior to first ovulation. This novel finding raises the question of whether there is a developmental window of vulnerability during which exposure to chronically elevated

Figure 2 Percentage of total births categorized as stillbirths by ovulatory status at MPA-treatment onset. Females placed on MPA treatment prior to first ovulation had a significantly higher incidence of stillbirth after MPA-treatment cessation than did females placed on MPA treatment after first ovulation. When controlling for dam, diabetes, and total drug exposure, ovulatory status at treatment onset was still a significant predictor of stillbirth, with exposure to MPA prior to first ovulation being positively associated with stillbirth (Table 5). * $P < 0.05$.

Table 5 Summary of nested binary logistic regression model predicting stillbirth.

Predictors	<i>B</i>	S.E.M.	Wald	<i>P</i>
Constant	6.11	3.10	3.90	0.05
Diabetes	2.85	1.35	4.48	0.03*
Ovulatory status at first treatment	−3.81	1.05	13.29	<0.01*
Total drug exposure	<0.01	<0.01	3.34	0.07

The dependent variable in this analysis is stillbirth coded so that 0=no and 1=yes. Ovulatory status at first treatment is coded so that 0=pre-ovulatory and 1=ovulatory. Diabetes is coded so that 0=no diabetes and 1=diabetes. *Significant at the level of $P<0.05$.

levels of progestin permanently and negatively alter future reproductive capacity.

Maternal abuse/neglect

The adolescent-treated females were more likely to abuse and/or neglect their infants than were their age-matched and parity-matched controls. Although we do not know the cause, this high incidence of aberrant maternal behavior in the adolescent-treated females may reflect a lack of pre-adult experience with infants. All adult females living in the social group containing the adolescent-treated females were placed on MPA treatment while the adolescent-treated females were still juveniles; consequently, these females had no opportunity to interact with infants until they gave birth to their own.

Return of fertility and reproductive output

The treated females showed considerable individual variation in latency to resumption of ovulation after their final MPA injections. Consistent with findings from studies of human MPA users (Pardthaisong *et al.* 1980, Lan *et al.* 1984, Garzes-Flores *et al.* 1985), latency to resumption of ovulation was seemingly unrelated to reproductive experience prior to treatment, weight at last treatment, or total drug exposure. Women show considerable variation in the rate at which they metabolize MPA (Schwallie & Assenzo 1974, Fotherby *et al.* 1980), and individual differences in MPA metabolism and clearance were likely responsible for the observed variation in latency to resumption of ovulation after the final MPA injection.

We found no evidence that MPA exposure negatively impacted post-treatment rates of conception for either adult- or adolescent-treated females. On average, the adult-treated females gave birth as often as did their matched controls during the study period, and the adolescent-treated females actually gave birth more often than did their matched controls. These results are consistent with findings from studies of human MPA users, in which rates of conception have been shown not to differ between former MPA users and age-matched controls (Schwallie & Assenzo 1974). The increased

reproductive output of the adolescent-treated females was likely related to their increased incidence of infant mortality. Female primates experience lactational amenorrhea, which lengthens the interbirth interval (Schallenberger *et al.* 1981, Stewart 1988, Recabarren *et al.* 2000). The death of an infant removes lactational amenorrhea, and mothers who lose an infant typically conceive again more quickly than do mothers who successfully wean an infant; therefore, more frequent infant loss increases reproductive output but not reproductive success. When interpreting the effects of MPA exposure on future fertility, it is crucial to distinguish between the ability to conceive and the ability to successfully reproduce. The current literature emphasizes post-treatment conception rates for human MPA users but neglects to investigate pregnancy outcomes following conception.

Speculations

We can only speculate as to why exposure to MPA during early adolescence might increase the risk of stillbirth in adulthood in primates. One possibility is that adolescent MPA exposure alters the structure of the adult uterus. In primates, the uterus continues to develop into puberty, and this postnatal uterine development is sensitive to progestins (Yin & Ma 2005). Evidence suggests that exposure to elevated levels of progestins during species-specific sensitive periods of uterine development can permanently alter adult uterine structure (for review, see Gray *et al.* 2001). In the current study, it is possible that exposure to MPA during early adolescence, a critical period of uterine development, permanently altered the structure of the uterus and increased the likelihood of stillbirth in adulthood in the adolescent-treated females.

A second possibility is that adolescent MPA exposure disrupts the oxytocin system. Oxytocin has been shown to be critical for the upregulation of uterine contraction strength during labor and the completion of parturition and for the expression of species-typical maternal behavior in a variety of mammalian species (Keverne & Kendrick 1992, Pedersen *et al.* 1992, Olazabal & Young, 2006). Therefore, a disruption of the oxytocin system could account for both the increased incidence of stillbirth and the increased incidence of aberrant maternal behavior in the adolescent-treated females. Progestins suppress uterine contractility during pregnancy, in part, by binding and blocking the uterine oxytocin receptor (OTR; Grazzini *et al.* 1998). Because progestins show an affinity for OTR, exposure to MPA during early adolescence could potentially disrupt the oxytocin system. Whether MPA exposure during adolescence is associated with decreased levels of circulating oxytocin during labor and/or following parturition, or decreased expression of OTR in the brain and/or uterus in adulthood, remains to be explored.

Conclusions

The findings of the present study point to a relationship between MPA exposure during early adolescence and an increased likelihood of stillbirth in adulthood, years after MPA treatment has ended. These findings suggest that MPA exposure during early adolescence may permanently decrease post-treatment reproductive capacity, particularly for females placed on MPA treatment prior to first ovulation. The Depo-Provera product insert states that Depo-Provera should not be used prior to menarche; however, the insert fails to address the use of Depo-Provera after menarche but prior to first ovulation (Pfizer Canada Inc. 2013). 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 MPA to adolescent girls.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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