Impact of antenatal selective serotonin reuptake inhibitor exposure on pregnancy outcomes in mice

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OBJECTIVE: This study investigates fluoxetine (FLX) exposure as an etiology for altered gestational length and adverse pregnancy outcomes.

STUDY DESIGN: Two experiments were performed exposing mice to drinking water (H_2O) or H_2O+FLX. Primary outcomes included gestational length, litter size, and live birth rate. In experiment 1, time-mated dams were monitored for spontaneous birth, and gestational length was calculated. In experiment 2, dams were dissected on day 14 to verify litter size and qualities of embryo implantation.

RESULTS: There was no difference in gestational length between H_2O dams (480.7 ± 13.2 hours) and H_2O+FLX dams (483.5 ± 10.1 hours), $P = .70$. Mean litter size was decreased in H_2O+FLX dams (4.1 ± 1.3/litter) compared to H_2O dams (5.5 ± 1.9/litter), $P = .04$. H_2O+FLX dams were less likely to have live births (25.4%) compared to H_2O dams (49.3%), $P = .01$.

CONCLUSION: Antenatal FLX exposure did not statistically alter gestational length, but did affect litter size and spontaneous loss in mice. This warrants further investigation.

Key words: birth outcomes, mouse model, pregnancy, preterm birth, selective serotonin reuptake inhibitor

Clinical depression is a common condition among women of reproductive age. Between 14-23% of pregnant women will experience a depressive disorder during pregnancy.[1] Although there is no standard screening protocol, prenatal depression is one of the most frequently encountered medical complications during pregnancy. The trend of antidepressant use during pregnancy has continued to rise over the last decade largely as a result of the increased use of selective serotonin reuptake inhibitors (SSRIs).[2,3] In 2003, approximately 13% of women took an antidepressant at some point in pregnancy, a rate that has doubled since 1999.[4]

While several research studies have focused on the potential maternal and fetal risks of SSRI use in pregnancy, including possible teratogenic effects, other studies have suggested that the increase in SSRI use may also affect prematurity rates.[5-7] The rate of preterm birth in the United States is approximately 12.7% of live births, which is an increase of >15% in the last decade.[8] This study will focus on the effect of SSRI exposure as an etiology for preterm birth in a mouse model.

Conflicting data exist linking SSRI use to an increased risk for preterm birth, shortened gestational length, or spontaneous fetal loss.[9-15] While Chambers et al[15] showed that third-trimester SSRI exposure was associated with higher rates of premature delivery, Malm et al[16] published a conflicting population-based study of pregnant women exposed to SSRIs vs matched controls finding no increased risk of preterm birth. Two meta-analyses have demonstrated that women exposed to SSRIs in the first trimester had higher rates of spontaneous abortions when compared to a group of women who were not exposed.[17,18]

Deficiencies within the literature exist demonstrating an association between antenatal fluoxetine (FLX) exposure and adverse pregnancy outcomes. Historically, studies have not accounted for obstetric factors relating to preterm birth, namely, history of a previous preterm birth or prior preterm premature rupture of membranes; nor have they differentiated among a spontaneous, indicted, or elective preterm birth. While these and many other factors are difficult to control in human studies, causal associations between FLX exposure and shortened gestational length can be approached using animal models. Accordingly, this study was initiated to study the impact of antenatal FLX exposure in a mouse model and to begin investigations of mechanistic pathways if an association was confirmed.

Materials and Methods

Two prospective, blinded observational experiments were performed. In experiment 1, 52 virgin female 129/SvEvTac mice (Taconic Farms, Germantown, New York) were time-mated (2 females and 1 male per cage) over 4 hours just prior to the end of the daily light cycle. Each female was examined for the presence of an ejaculatory plug in the vagina.

TABLE
Pregnancy outcomes

Outcome	Experiment 1 (n = 52)			Experiment 2 (n = 76)			Total (n = 128)		
	Drinking water only (n = 29)	Drinking water plus fluoxetine (n = 23)	P	Drinking water only (n = 40)	Drinking water plus fluoxetine (n = 36)	P	Drinking water only (n = 69)	Drinking water plus fluoxetine (n = 59)	P
Length of gestation, h	480.7 ± 13.2	483.5 ± 10.1	.70	NA	NA	NA	NA	NA	NA
Mean weight gain at gestational day 14, g	6.1 ± 2.7	4.9 ± 1.3	.44	6.1 ± 2.4	4.9 ± 1.0	.12	6.0 ± 2.5	5.2 ± 1.2	.27
Live birth rate (confirmed pregnancy/plugged)	44.8% (13/29)	17.4% (4/23)	.04	52.5% (21/40)	30.6% (11/36)	.05	49.3% (34/69)	25.4% (15/59)	.01
Mean litter size (pups/litter)	5.8 ± 1.7	4.5 ± 1.7	.22	5.5 ± 1.9	4.1 ± 1.3	.02	5.5 ± 1.9	4.1 ± 1.3	.04
Missed abortion rate	NA	NA	NA	0.0% (0/21)	18.2% (2/11)	.20	NA	NA	NA

NA, not applicable.

Bauer. Antenatal SSRI exposure impact on pregnancy outcomes in mice. Am J Obstet Gynecol 2010.

The plug, composed of coagulated secretions from male accessory sex glands, indicated that coitus and ejaculation had occurred. Dams were randomly assigned to 1 of 2 cohorts. Group 1 (n = 29) had continuous access to drinking water (H_2O) only, while group 2 (n = 23) had access to H_2O+FLX. Dams were monitored every 12 hours for spontaneous birth and length of gestation, which were recorded and analyzed. The individual cages were monitored for the date and time of birth every 4 hours starting on day 18. The mouse dams and pups were utilized in subsequent experiments, independent of this study, and ultimately sacrificed.

In experiment 2, 76 virgin female 129/SvEvTac mice (Taconic Farms) were mated (2 females and 1 male per cage) just prior to the end of the daily light cycle. After plug confirmation, dams were randomly assigned to 1 of 2 cohorts. Group 1 (n = 40) had continuous access to H_2O, while group 2 (n = 36) had access to H_2O+FLX. Plugged dams were sacrificed, dissected, and analyzed on day 14 following mating to verify pregnancy and assess for litter sizes and qualities of embryo implantation. H_2O or H_2O+FLX was initiated after plug confirmation and continued until the end of pregnancy. FLX was dissolved in H_2O at the dose of 4 mg/50 mL to achieve an approximate 10 mg/kg/d dosing. FLX was freely available in the animal's H_2O in opaque bottles to protect from light and changed weekly. FLX was chosen because it is the most commonly prescribed antidepressant during pregnancy and because of its extended half-life. The dosing regimen utilized by our laboratory and others has repetitively produced therapeutically relevant blood levels in mice. FLX and the metabolite levels have been measured in serum and whole brain and serum, and it has been previously shown that steady state for both FLX is achieved by 5 days of receiving FLX in the H_2O (10 mg/kg/d), as serum levels after 5 or 90 days of treatment are equivalent. FLX levels were determined by liquid chromatography with fluorescence detection.[19,20] Detection of a copulatory plug designated gestational day and time 0. The mated mice were housed individually and were weighed weekly and at the time of delivery. The average length of gestation of a 129/SvEvTac mouse is 480 hours, and the average 129/SvEvTac dam produces a litter size of 6.4 ± 0.88 pups (Taconic Farms).

Animals were maintained in a 12-hour light and dark cycle in a temperature- and humidity-controlled environment. Unlike the animal care technicians, the individuals observing the pup deliveries were blinded and unaware of the composition of the subjects' H_2O. The animals were fed with standard food pellets. Animal testing was conducted in accordance with the National Institutes of Health laboratory animal care guidelines and with Columbia University Institutional Animal Care and Use Committee approval.

Based on pilot data, a power analysis was performed, with an alpha of 0.05, and a beta of 0.80, and it was determined that a minimum of 21 dams would be needed in each group for each experiment to demonstrate a decrease in gestational age by 1.1 days or 5%, for a total sample size of 42 dams. The live birth rate was calculated as the ratio of pregnant dams to the total number of plugged dams. The data are expressed as mean ± SD. The significance of differences among the groups was assessed using 1-way analysis of variance test and Fisher's exact test. The criterion for significance for all analyses was $P \leq .05$. The criterion for a trend was $.05 < P < .1$.

Results

The Table presents the findings from experiment 1 (length of gestation), including differences in mean weight gain at gestational day 14 and mean litter size. In all, 52 females showed evidence of vaginal plugs following the brief exposure to males. Of these, 17 dams ultimately had live births. There was no significant difference in gestational length between H_2O dams (n = 13; 480.7 ± 13.2 hours) and H_2O+FLX dams (n = 4; 483.5 ± 10.1 hours), $P = .70$. However, dams exposed to H_2O+FLX were less likely to have live births, 17.4% (4/23), than dams exposed to H_2O alone, 44.8% (13/29), $P = .04$.

For experiment 2 (litter size and embryo quality), 76 mice were confirmed to

be pregnant by presence of a vaginal copulatory plug. Mean litter size was decreased in H_2O+FLX dams (4.1 ± 1.3/litter) compared to H_2O dams (5.5 ± 1.9/litter), $P = .02$. Live birth rates were decreased in dams exposed to H_2O+FLX, 30.6% (11/36), compared to dams exposed to H_2O alone, 52.5% (21/40), $P = .05$. While not statistically significant, uterine horn dissection to assess for nonviable pregnancy tissue or missed abortion revealed an 18.2% (2/11) rate in H_2O+FLX dams compared to a 0.0% (0/21) rate in H_2O dams, $P = .20$. The mean weight gain at day 14 in H_2O+FLX dams (4.9 ± 1.0 g) compared to H_2O dams (6.1 ± 2.4 g) did not reach statistical significance, $P = .12$.

Given the identical experimental design, data between experiments 1 and 2 were combined to increase statistical power. For the combined dataset, live birth rates were decreased in dams exposed to H_2O+FLX, 25.4% (15/59), compared to dams exposed to H_2O alone, 49.3% (34/69), $P = .01$. Mean litter size was decreased in H_2O+FLX dams (4.1 ± 1.3/litter) compared to H_2O dams (5.5 ± 1.9/litter), $P = .04$. The mean weight gain at day 14 in H_2O+FLX dams (5.2 ± 1.2 g) was decreased when compared to H_2O dams (6.0 ± 2.5 g); however, this finding was not statistically significant.

COMMENT

Length of gestation, the primary outcome, was not affected by FLX exposure when compared to H_2O alone in a mouse model; however, FLX exposure did affect litter size and spontaneous fetal loss. Significant findings within this study include the decrease in live birth rate and in the mean litter size in dams receiving H2O+FLX compared to dams receiving H2O. These findings potentially suggest a physiological alteration at the placenta level during implantation and warrant further investigation. Current available data on adverse pregnancy outcomes following antenatal exposure to SSRIs, including spontaneous abortion and preterm birth, in both human beings and animals, remain contradictory. Several human studies support SSRI exposure as an etiology for adverse pregnancy out-

comes. Sparse literature from animal models is available for comparison. Bairy et al[21] evaluated FLX exposure in rats and concluded that there was no significant alteration in the gestation period, and reported no significant alterations in litter size or birthweight.

The serotonin system includes at least 14 receptors, multiple enzymes, and transporter proteins that exert influence on serotonin metabolism, release, and reuptake.[22] SSRIs actively pass through the placenta and their metabolites have been detected in roughly equivalent values in both umbilical cord blood and amniotic fluid.[23] Three plausible biological pathways for adverse pregnancy outcome as a result of antenatal SSRI exposure are SSRI-associated increases in cortisol, increases in estriol levels, and uterine artery vasoconstriction.

The first mechanism involves maternal cortisol levels, which have been shown to predict the rise in corticotropin-releasing hormone (CRH), providing further evidence of how stress hormones may work in concert to lead to early parturition. SSRIs influence the hypothalamic pituitary-adrenal axis feedback mechanisms, which also change during pregnancy. Glucocorticoids during pregnancy have been shown to cause a progressive 20-fold increase in CRH levels across the course of pregnancy. Hobel[24] demonstrated that CRH levels increased more dramatically in women at risk for preterm delivery. FLX infusion has been associated with an additional increase in the magnitude of the normal antenatal rise in fetal plasma CRH, adrenocorticotropic hormone, and cortisol concentrations.[25]

Next, salivary estriol has been shown to be a marker for preterm birth. Suri et al[26] measured salivary estriol, a hormone that increases exponentially in the few weeks before the onset of spontaneous term and preterm labor, in pregnant women with and without antidepressant treatment. Prenatal antidepressant use was associated with significantly higher salivary estriol levels in the second half of pregnancy. Whether estriol reflects a causal mechanism by which women on antidepressants have shorter pregnancy duration remains to be further studied.[27]

A final proposed mechanism relates to the effect of FLX on plasma serotonin. FLX prolongs exposure to endogenously released serotonin, a known potent uterine vasoconstrictor, thus leading to a transient reduction in uterine blood flow. This, in turn, would reduce the delivery of oxygen and nutrients to the fetus, thereby presenting a mechanism for reducing growth and/or eliciting preterm delivery.[28] Following administration of FLX to pregnant ewes, Morrison et al[29] reported a transient decrease in uterine artery blood flow, fetal partial pressure of oxygen and oxygen saturation.

One strength of this study includes the degree of precision to which the timed mating was performed. Day 0 of gestation was estimated within 4 hours thereby providing a robust method for studying the antenatal exposure of FLX as an etiology of adverse pregnancy outcomes. In addition, by calculating a sample size, statistical significant results can be confidently reported. This study provides a novel method for studying the antenatal exposure of FLX as an etiology of adverse pregnancy outcomes. Studies have shown that placental transfer of FLX is similar between mouse and human.[30] Despite the advantage of targeted experiments in the mouse model and similarities in placental cell types between the mouse and human, the animal model might be limiting in understanding all aspects of the effects of antenatal SSRI exposure in the physiology of mouse and human pregnancies.[31] The mode of implantation differs between mouse and human; there is shallow decidual invasion in mouse compared to extensive uterine remodeling by invasive trophoblasts in human. In addition, the uterine spiral arterioles in mice are remodeled by maternal factors rather than by invasive trophoblasts in human pregnancies.[32] Moreover, the short gestational period in mice and incomplete development at birth are unlike human pregnancies.

In conclusion, we found that mice exposed to FLX had similar gestational lengths, but FLX exposure did affect litter size and spontaneous loss. Whether FLX exposure induces these results on pregnancy outcome independently re-

mains unclear. These findings warrant additional investigation as they point to a significant impact on pregnancy outcomes associated with antenatal FLX exposure. Studies of the effects of serotonin at the placental level at the time of pregnancy implantation may also provide evidence for some of the clinical outcomes identified in this study and seen in the clinical literature. ∎

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