ARE SSRI'S EQUALLY SAFE IN PREGNANCY? FLUOXETINE AND CITALOPRAM EXPERIMENTAL STUDY

BIANCA EUGENIA ÖSZ[1], CAMIL EUGEN VARI[1*], MELINDA KOLCSÀR[1], OVIDIU SIMION COTOI[2], MARIUS ŞTEFAN MĂRUŞTERI[3], MARIA T. DOGARU[1]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Medicine and Pharmacy, Târgu Mureş
[2]Department of Cellular and Molecular Biochemistry, Faculty of Medicine, University of Medicine and Pharmacy, Târgu Mureş
[3]Department of Medical Informatics and Biostatistics, Faculty of Medicine, University of Medicine and Pharmacy, Târgu Mureş
** corresponding author: camil.vari@umftgm.ro*

Abstract

The aim of the present study was to determine how fluoxetine and citalopram are affecting gestation and fetal development in Wistar rats. Forty sexually mature female rats, divided in two experimental groups received 20 mg/kg body weight fluoxetine or 3 mg/kg body weight citalopram during the entire gestation period starting day one. Drugs were administered by oral route. A control group (20 females) received distilled water by oral gavage in the same conditions. Healthy males were caged with nulliparous females for an entire week. All females were killed with halothane through gas scavenging apparatus untilled death on assumed day 21 of gestation for examination of their uterine contents. The number of healthy foetuses, resorbtions and dead foetuses were noted. In both experimental groups resorbtion occurred and dead foetuses were identified. The resorbtions were confirmed by histopathological analyses. The statistical analysis of results showed that only citalopram is affecting gestation in a significant manner ($p<0.01$). For fluoxetine the number of resorbtions and death foetuses was not significant different compared with control group ($p>0.05$).

Rezumat

Experimentul a avut drept scop evaluarea influenței fluoxetinei şi a citalopramului asupra gestației, medicația administrându-se în paralel. S-au utilizat două loturi de animale alcătuite din câte 20 de şobolani albi Wistar, de sex feminin. Primul lot a fost tratat cu fluoxetină în doză de 20 mg/kg corp, iar cel de-al doilea lot cu citalopram, 3 mg/kg corp pe toată perioada gestației. Un alt treilea lot, alcătuit din 20 femele gestante a servit drept martor. Medicația a fost administrată prin gavaj. Masculii au fost ținuți împreună cu femelele, nulipare, timp de o săptămână, după care au fost separați. În ziua 21 de gestație femelele au fost sacrificate prin administrarea unei supradoze de anestezic general (halotan inhalator) după care uterul a fost analizat macroscopic. S-a urmărit: numărul puilor vii, morți, respectiv numărul resorbțiilor uterine în cazul fiecărui individ. În cazul loturilor tratate medicamentos au putut fi identificate pe lângă fetuşi vii, resorbții uterine şi fetuşi morți. Resorbțiile uterine au fost confirmate de analiza histopatologică. Analiza statistică a rezultatelor sugerează faptul că doar în cazul lotului tratat cu citalopram

există diferențe statistic semnificative în ceea ce privește numărul de resorbții, respectiv fetuși morți comparativ cu lotul martor ($p<0,01$).

Keywords: fluoxetine, citalopram, rat, resorbtion, dead embryos.

Introduction

Depression during pregnancy occurs in 10% of women and the treatment is essential to prevent premature birth, low birth weight or other problems for offspring's. The use of antidepressants during pregnancy is based on the balance between risks and benefits but few medications have been proved to be safe.

Of all antidepressants the Guideline's and FDA (Food and drug Administration) recommends in pregnancy the use of SSRI's (selective serotonin reuptake inhibitors).

Materials and Methods

Sixty pregnant female Wistar rats were divided in three groups: Group 1 received 20 mg/kg body weight fluoxetine, Group 2 - 3 mg/kg body weight citalopram and Group 3 – saline solution during the entire gestation period starting day one. Drugs were administered by oral route. The animals were obtained from the University of Medicine and Pharmacy of Târgu Mureş Biobase. Healthy, nulliparous animals were selected along with 20 female Wistar rats, as control group. Animals were kept under the same condition of temperature and humidity and received standard rat diet and fresh drinking water *ad libitum*.

Animal experiments were made in concordance with Directive 2010/63/EU of the European Parliament and of the Council regarding protection of animals used for scientific purpose and approved by the Ethical Committee of University of Medicine and Pharmacy of Târgu Mureş.

Two males were kept with 4 females for an entire week. Female rats have a regular 4-day oestrous cycles ovulation but ovulation can also occur spontaneously in the presence of male. After copulation the females were relocated into separate cages until parturition.

All females were killed with halothane through gas scavenging apparatus on assumed day 21 of gestation for examination of their uterine contents. At necropsy, the ovaries and uterus were removed and examined. Pregnancy status, number and distribution of alive foetuses and embryonic/fetal deaths were noted. Embryonic deaths were classified as either early (only embryonic tissue visible) or late (both the placenta and

embryonic tissue were visible). Each foetus was examined for external defects.

Uterine tissue samples were collected and fixed in 10 % neutral buffered formalin for *histopathological examinations*. The samples were embedded in paraffin wax, and were cut into 5 μm sections and stained with haematoxylin and eosin. The histological sections were examined for the presence of embryonic tissues or abnormal cells using a microscope.

The data are unpaired (independent samples). Thus, for *statistical interpretation* of data's, descriptive statistics was performed, and then a normality test was applied (Kolmogorov–Smirnov test - KS test).

Based on the results of the normality test, a parametric One-Way Anova or their non-parametric equivalents (The Kruskal –Wallis – KW test) were chosen.

The Dunn's test was used as a *post-hoc* test (which primarily compares the control *versus* experimental groups), for multiple comparisons: control group/fluoxetine group, control group/citalopram group, then citalopram group/fluoxetine group. The analyses were performed using *GraphPad Instat Software* [1].

Results and Discussion

In order to draw a valid conclusion of SSRI's (selective serotonin reuptake inhibitors) influence on embryos and foetuses the examination of rat uterus was analysed macroscopically and microscopically. The data was then subjected to statistical analysis.

Macroscopic examination of uterine contents:

In the 21 day of gestation caesarean dissection was done and the ovaries and uterus were removed and examined. Pregnancy status, the number and distribution of live foetuses and embryonic/fetal deaths were noted.

The number of healthy foetuses, resorbed embryos and dead foetuses for each group are presented in Table I.

Table I

Comparative distribution of resorbed embryos and live or dead foetuses among the three animal groups

	Live foetuses	Resorbed embryos	Dead foetuses
Control group	146	0	0
Fluoxetine group	141	24	4
Citalopram group	41	56	2

In the control group the caesarean dissection revealed an increase volume of the uterine horns and the foetuses could be noted and appreciated by transparency. They had a normal, symmetrical distribution in the uterine horns (Figure 1 – A) and a normal exterior aspect (Figure 1 – B).

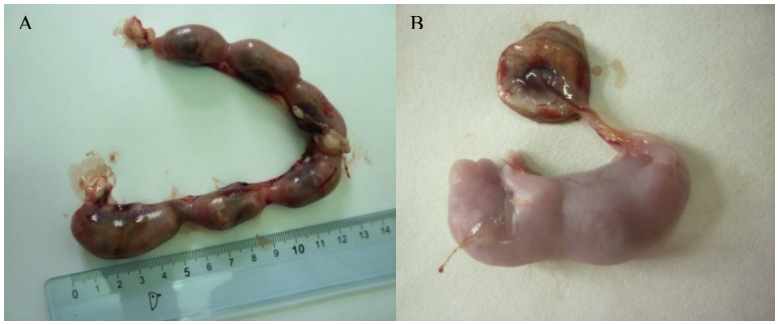

Figure 1.
Normal uterine horn with foetuses (A) and normal foetus (B)

In the fluoxetine and citalopram treated groups the uteri examination revealed the presence of resorbtions as seen in Figure 2 – B.

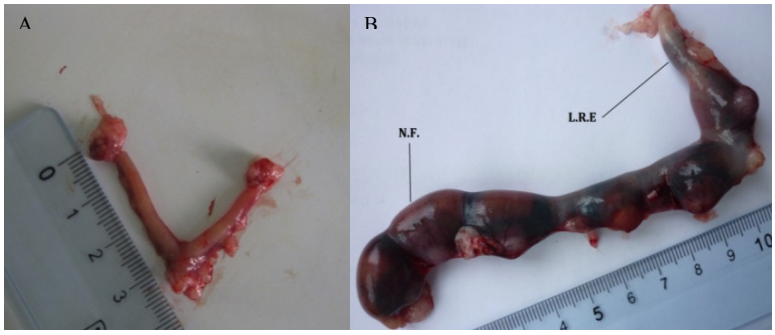

Figure 2.
Normal uterus (A), uterus with live foetuses (N.F.) and late resorbed embryos (L.R.E.) - citalopram

Small heaps were also noted in some animals. These heaps correspond to the reabsorbed embryos. In Figure 3 – A in both uterine horns resorbed embryos can be seen.

There were also females in which only the placental rest was found therefore it was concluded that resorbtion occurred in a late stage of development. In figure 3 – B, there are 3 live foetuses and one early resorbed embryo in one uterine horn and in the other horn there were no signs of implantation.

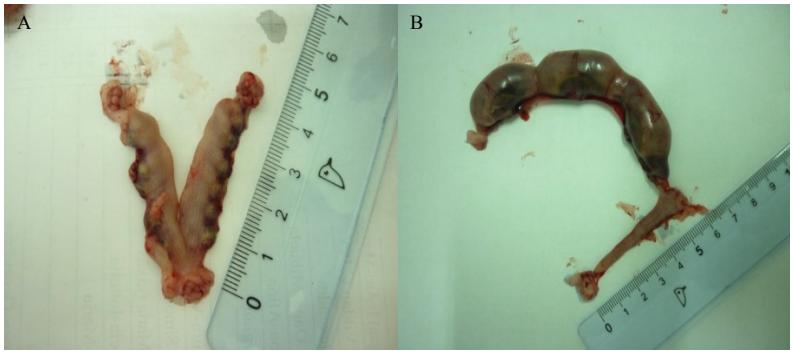

Figure 3.
Late resorbed embryos (A) and horn with no implantation sites (B)

Histopathological analysis:

After they were fixed in 10% neutral buffered formalin the uterine tissue samples were embedded in paraffin wax, cut into 5 µm sections and stained with haematoxylin and eosin. The histological sections were examined for the presence of embryonic tissues or abnormal cells with a microscope.

The histopathological analyses confirmed that resorbtions occurred. Microscopic examination revealed distinct areas of embryonic tissue remands - Figure 4.

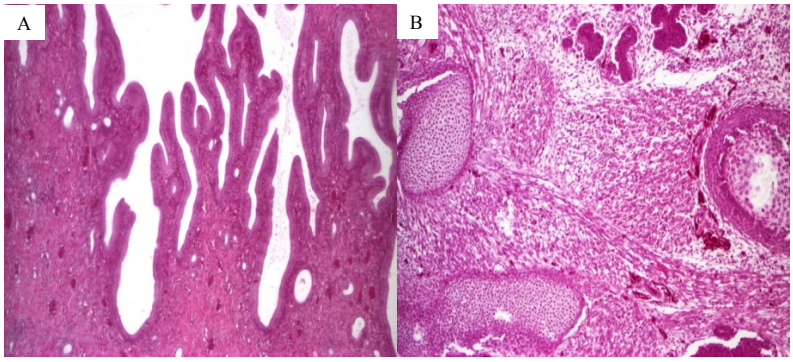

Figure 4.
Empty uterine cavity - the microscopic appearance of rat endometrium (A) and embryonic tissue remands in uterine cavity (B). (Haematoxylin-eosin staining, Ob.2x)

Statistical analysis:

The statistical analysis was performed considering the total number of events (resorbtions and dead foetuses), by comparing the average/mean

(for a parametic test) or median (for the non-parametric test) number of resorbtions and fetal deaths with the control group. The values were calculated per each female rat (total number of resorbtions or dead foetuses for each female rat).

Descriptive statistics was made, and then a normality test was applied (Kolmogorov–Smirnov test - K–S test). The experimental data's did not reached criteria for parametric statistical analysis, so the non-parametric test Kruskal–Wallis (K-W test) was applied because the number of accidents in the control group was null. Anyway, by using ranks instead of simple means comparisons, a non-parametrical statistical test is more suitable for a comparison with the zero values observed in the control group (where, obviously, standard deviation is also zero).

Then, the Dunn test was consequently used as *post-hoc* test, in order to compare the control groups with both experimental groups. The analyses were made using *GraphPad Instat Software*.

There was a statistically significant difference between medians of the number of accidents between the control group and citalopram group ($p<0.001$), but no statistically significant difference between control group and fluoxetine group ($p>0.05$), respectively between the two experimental groups ($p>0.05$).

Conclusions

There are few preclinical studies that have examined the effect of SSRI's (selective serotonin reuptake inhibitors) during gestation. The effects of antidepressants on the embryo and foetus are still uncertain. Several SSRIs are generally considered an option during pregnancy, including citalopram and fluoxetine.

The potential risk of antidepressants during pregnancy varies. Some research studies associated the use of citalopram and fluoxetine with persistent pulmonary hypertension of the newborn when taken during the last half of pregnancy, as well with lower birth weight or mild nonsyndromic heart defects (ventricular septal defect, bicuspid aortic valve and right superior vena cava to coronary sinus) when taken in the first trimester of pregnancy [2, 3, 4] .

The prospective studies made on SSRI's are not in accordance with these results. SSRI's were not associated with either increased risk for major malformations or higher rates of miscarriage, stillbirth, or prematurity in prospective studies [5, 6].

There are also reports of withdrawal symptoms in newborns exposed to antidepressants in the last few weeks of pregnancy. These may include mild breathing problems, irritability, difficulty in settling and feeding.

The present study revealed that both citalopram at 3 mg/kg body weight and fluoxetine at 20 mg/kg body weight affect the embryo and fetal development in Wistar rats. In both treated groups resorbtions occurred. The uteri examination revealed only placental rest in some females, which suggest that resorbtion occurred in the late stage of gestation but there were also embryonic remands in uterine horns which correspond to late resorbtions. The presence of embryonic remands in uterine horns was confirmed by histopatological analysis.

The statistical analysis confirmed that citalopram affects gestation, the number of accidents (resorbtions and death foetuses) being statistically different compared with the control group. In fluoxetine treated group the number of accidents was not statistically different compared with the control group. In the case of fetal deaths there were no statistically significant differences between groups.

For fluoxetine, other preclinical studies have revealed fetal growth retardation and skeletal malformations in mice [6]. Another study made on rats and rabbits did not reveal any reproductive toxicity for fluoxetine doses that were maternally toxic [8].

In clinical studies fluoxetine exposure during the first trimester of pregnancy was not associated with significant teratogenic effects [9]. The incidence of congenital malformation in neonates exposed to fluoxetine during the first trimester was comparable to those exposed to either nonteratogens or tricyclic antidepressants. However women treated with fluoxetine had a tendency for increased risk for miscarriage when compared with women exposed to nonteratogens [10]. Another multicentre, prospective, controlled study which evaluated the rate of major congenital anomalies after first-trimester gestational exposure to fluoxetine (314 fluoxetine first-trimester exposed pregnancies) or nonteratogens (1467 controls) suggested a possible association between cardiovascular anomalies and first-trimester exposure to fluoxetine [11].

For citalopram pregnant Wistar rats' exposure in doses of 10 and 20 mg/kg body weight/day affected the phagocytic cell population of fetal liver [12].

A clinical study (retrospective cohort study) suggested that citalopram use can be associated with neural tube defects [13].

This study revealed that in rats, the use of fluoxetine during gestation period is much safer than citalopram administration, but to allow a proper conclusion about SSRIs use in pregnancy further research is needed.

References

1. Măruşteri M., Bacârea V., Comparing groups for statistical differences: how to choose the right statistical test? *Biochemia Medica*, 2010; 20(1): 15-32.
2. Addis A., Koren G., Safety of fluoxetine during the first trimester of pregnancy: a meta-analytical review of epidemiological studies. Psychol. Med., 2000; 30: 89-94.
3. Tuccori M., Montagnani S., Testi A., Ruggiero E., Mantarro S., Scollo C., Pergola A., Fornai M., Antonioli L., Colucci R., Corona T., Blandizzi C., Use of selective serotonin reuptake inhibitors during pregnancy and risk of major and cardiovascular malformations: an update. *Postgrad. Med*., 2010 Jul; 122(4): 49-65.
4. Kieler H., Artama M., Engeland A., Ericsson O., Furu K., Gissler M., Nielsen R.B., Nørgaard M., Stephansson O., Valdimarsdottir U., Zoega H., Haglund B., Selective serotonin reuptake inhibitors during pregnancy and risk of persistent pulmonary hypertension in the newborn: population based cohort study from the five Nordic countries. *B.M.J*., 2012 Jan 12; 344: d8012.
5. Kulin N.A., Pastuszak A., Sage S.R., Schick-Boschetto B., Spivey G., Feldkamp M., Ormond K., Matsui D., Stein-Schechman A.K., Cook L., Brochu J., Rieder M., Koren G., Pregnancy outcome following maternal use of the new selective serotonin reuptake inhibitors: a prospective controlled multicenter study. *J.A.M.A*., 1998 Feb 25; 279(8): 609-610.
6. Goldstein D.J., Corbin L.A., Sundell K.L., Effects of first-trimester fluoxetine exposure on the newborn. *Obstet. Gynecol*., 1997 May; 89(5 Pt 1): 713-718.
7. Merlob P., Birk E., Sirota L., Linder N., Berant M., Stahl B., Klinger G., Are selective serotonin reuptake inhibitors cardiac teratogens? Echocardiographic screening of newborns with persistent heart murmur. Birth Defects. *Res. A. Clin. Mol. Teratol*., 2009 Oct; 85(10): 83741.
8. Byrd R.A., Markham J.K., Developmental toxicology studies of fluoxetine hydrochloride administered orally to rats and rabbits. *Fundam. Appl. Toxicol*., 1994 May; 22(4): 511-518.
9. Nicolae A.C., Mitrea N., Drăgoi C.M., Constantinescu M.Z., Ciofrângeanu C., Bărboi G., Arsene A. L. Murine studies regarding the variation of oxidative status in serum, hepatic and brain samples, after administration of some CNS active drugs. *Farmacia*, 2013; 61(4): 658-669.
10. Pastuszak A., Schick-Boschetto B., Zuber C., Pregnancy outcome following first. Trimester exposure to Fluoxetine (Prozac®). *J.A.M.A*., 1993; 269: 2246-2248.
11. Diav-Citrin O., Shechtman S., Weinbaum D., Wajnberg R., Avgil M., Di Gianantonio E., Clementi M., Weber-Schoendorfer C., Schaefer C., Ornoy A. Paroxetine and fluoxetine in pregnancy: A prospective, multicenter, controlled, observational study. *Br. J. Clin. Pharmacol*., 2008; 66(5): 695-705.
12. Răducanu I., Segârceanu A., Prada G., Ionescu C., Arsene A., Fulga I., Assessing depressive effect of ketoprofen and its mechanism of action using the forced swimming test in mice. *Farmacia*, 2012; 60(5): 759-766.
13. Malm H., Artama M., Gissler M., Ritvanen A., Selective serotonin reuptake inhibitors and risk for major congenital anomalies. *Obstet. Gynecol*., 2011 Jul; 118(1): 111-120.

Manuscript received: December 2013