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Reproductive performance of pregnant rats and embryotoxic effects of ciprofloxacin

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Received July 12, 2005, accepted August 25, 2005

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Pharmazie 61: 79–80 (2006)

To observe the effect of ciprofloxacin on both pregnancy and physical development of fetuses, the drug was orally administered to thirty pregnant rats at doses of 20 and 40 mg/kg body weight from day-1 to day-19 of gestation. The intact rat fetuses were isolated on day-19 of gestation. Ciprofloxacin induced general changes in the reproductive performance of female rats and significant alterations in the development of the skeletal parameters of fetuses.

The use of drugs during pregnancy and breastfeeding demands a critical evaluation regarding exposure period, dose, therapy term and fetal and neonatal susceptibility (Gerenutti et al. 1991; Del Fiol et al. 2005).
One group of antimicrobial agents used to treat urinary infections that do not respond to conventional treatment is the quinolones. They are used because they usually reach high concentrations in the urinary tract (Hooton and Stamm 1997).

Table 1: Effects of ciprofloxacin on the reproductive performance of female rats (10 animals per group)

Parameters	Control	Ciprofloxacin 20 mg/kg	Ciprofloxacin 40 mg/kg
Pre-implantation loss (%)	1.07	3.51*	4.62*
Post-implantation loss (%)	0	3.63*	5.49*
Placenta weight (grams)	0.63 ± 0.15 (n = 92)	0.57 ± 0.02 (n = 106)	0.51 ± 0.15* (n = 86)
Fetuses' weight (grams)	2.42 ± 0.18 (n = 92)	2.47 ± 0.41 (n = 106)	2.39 ± 0.22 (n = 86)
Fetuses' vitality (%)	97.87	96.36	93.41

Data are reported in percentage and mean ± S.E.M.
*$p < 0.05$ (Chi-square test or Tukey-Kramer test)

Quinolones, such as ciprofloxacin, norfloxacin, gatifloxacin, levofloxacin and ofloxacin, are wide-spectrum bactericidal antibiotics that inhibit the DNA-gyrase of bacteria (Saravanos and Duff 1992). These agents reach high concentrations in the amniotic fluid and umbilical cord blood (Loebstein et al. 1998). However, both mammalian and bacterial gyrases are very similar, raising great concern to the use of these agents during pregnancy. Some studies carried out in animals have shown damage to fetal cartilages (Grady 2003) or severe arthropathies in the offspring (Yabe et al. 1997; Nagai et al. 2002) when mothers were exposed to quinolones.
Despite serious adverse effects observed in animal models, other studies in human beings did not find alterations in the joints of babies from mothers exposed to quinolones in different periods of pregnancy (Berkovitch et al. 1994; Danisovicova et al. 1994; Loebstein et al. 1998). These antibiotics are relatively new in the global market and there is insufficient experience to guarantee safety to pregnant women and the fetuses. FDA classifies the quinolones as category C agents. Thus, the use of these agents in pregnancy should be avoided.

Table 2: Effects of ciprofloxacin on fetuses' external morphological parameters, fetuses' sternum ossification and fetuses' osseous structure (50 animals per group)

Parameters	Control	Ciprofloxacin 20 mg/kg	Ciprofloxacin 40 mg/kg
Measures of pupies (cm)	mean ± S.E.M	mean ± S.E.M	mean ± S.E.M
Skull – antero-posterior	1.16 ± 0.009	1.19 ± 0.07	1.03 ± 0.01*
Skull – latero-lateral	0.57 ± 0.050	0.60 ± 0.05	0.43 ± 0.02*
Thorax – antero-posterior	0.67 ± 0.005	0.72 ± 0.04	0.53 ± 0.02*
Thorax – latero-lateral	0.69 ± 0.050	0.70 ± 0.06	0.54 ± 0.02
Cranio-caudal length	3.21 ± 0.150	3.37 ± 0.16	2.98 ± 0.24
Tail length	0.59 ± 0.060	0.61 ± 0.07	0.50 ± 0.02
Number of Sternum Ossifications	% Puppies	% Puppies	% Puppies
0	18.0	11.0	29.0*
1	2.0	7.5	5.7
2	6.0	24.0 *	17.0*
4	40.0	26.0	46.0
5	34.0	30.0	2.9*
	% Puppies	% Puppies	% Puppies
Flattening on the skull soft tissues and bones	12.0	23.0*	46.0*

Data are reported in percentage and mean ± S.E.M.
*$p < 0.05$ (Chi-square test or Tukey-Kramer test)

The objective of the present study was to observe the effect of ciprofloxacin on the reproductive performance of female rats and on some morphological parameters of fetuses.

The results in Table 1 indicate that the administration of ciprofloxacin during pregnancy, in both doses (20 and 40 mg/kg), significantly increased the percentage of pre- and post-implantation losses and induced changes in placenta wet-weight. However, ciprofloxacin did not induce statistically significant differences in the body-weight and vitality of fetuses.

Table 2 shows that both ciprofloxacin doses (20 and 40 mg/kg) caused statistically significant alterations in the external measurements of the morphological parameters of the fetuses. A significative anticipation of the ossification period of the sternum, a flattening on the skull soft tissues and bones were also verified. However, other common anomalies such as syndactyly, cleft palate, and abnormal eyes/ears implantation were not observed.

The results of the present study indicate that, ciprofloxacin has promoted general alterations in the female-rats' reproductive performance and in the skeletal parameters of fetuses.

Experimental

The present study was approved by the University of Sorocaba Animal-study Ethics Committee. Male and female (*Rattus norvegicus albinus*, Wistar) rats, age 3 to 4 months, weighing 160 to 200 g, were used. In order to allow cohabitation, 2 males and 5 females were kept together overnight in plastic cages.

The first day of pregnancy was indicated by the presence of spermatozoids in the vaginal-washing smear observed at optical microscopy (Vickery and Bennett 1970). Thirty pregnant females were divided into three groups ($n = 10$). Two groups were exposed to 20 and 40 mg/kg ciprofloxacin administered orally (Galena Quality Control Laboratory, Sao Paulo, Brazil), respectively. The third group received deionized water (control group). Water and food were supplied *ad libitum* during all the experiment. The body-weight of all females was recorded daily. On the twentieth day of pregnancy, all animals were sacrificed and the ovaries were removed to count the *corpora lutea*. The uterus, placenta, and fetuses were also removed and the vitality of fetuses was recorded (Lemonica et al. 1996).

Previously macroscopic analyses, the fetuses were randomly divided into two groups after tissue fixation with Bouin's solution. A pachymeter was used to take measurements of the following: antero-posterior and latero-lateral skull lengths; antero-posterior and latero-lateral thorax lengths, cranio-caudal length, and tail length (Sterz and Lehmann 1985; Barrow and Taylor 1967). Evisceration and diaphanization of all fetuses were performed to study bones (Dawson 1926).

The fertility rate of females was calculated by using the following formulas:

$$\text{Pre-implantation losses} = \frac{\left[\begin{array}{c}\text{number of } \textit{corpora lutea} - \\ \text{number of implantations}\end{array}\right]}{\text{number of } \textit{corpora lutea}}$$

$$\text{Post-implantation losses} = \frac{\left[\begin{array}{c}\text{number of implantations} - \\ \text{number of alive fetuses}\end{array}\right]}{\text{number of implantations}}$$

The results were submitted to statistical analyses, considering a significance level of 5%. Tukey-Kramer test was used to compare experimental and control groups, considering body-weight gain, the reproductive performance of female rats (weights of placentas and fetuses) and all offspring morphological parameters. Chi-square test was used to evaluate changes (in percentage) of osseous development parameters, pre-implantation losses, post-implantation losses and fetuses' vitality.

Acknowledgements: The authors thanks for the assistance from: Mr. Jorge Valerio, Aline C. Farrapo, Lia B. Leite.

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