

LETTER TO THE EDITOR

ULTRASOUND IRRADIATION OF PREGNANT MICE

Sir,

This study was a follow-up of previous work [Fry *et al.*, 1978; Fry, 1986] with continuous wave (cw) and pulsed ultrasound exposure in the pregnant mouse. Those studies indicated that pulsed ultrasound of a specific spatial-peak temporal-average (SPTA) intensity did not appear to produce more fetal bioeffects when compared to cw exposure of the same average intensity. This new study was part of a larger study on the effect of nonlinear shock wave ultrasound fields on tissue, and supplies additional data comparing cw to pulse exposure.

Irradiation was conducted using a single 1 MHz fundamental focused beam having a half-intensity beamwidth of near 4.5 mm below 100 W cm<sup>-2</sup> [Fry *et al.*, 1989]. The specific details of the field configuration and calibration have been covered elsewhere [Fry *et al.*, 1989]. All details of the mice irradiation protocol have been previously reported [Fry, 1986]. The animals were irradiated on day 8 of gestation. The matrix of exposure sites in this study was different from the 4 mm spacing used previously. This study involved a 1 mm spacing distance for individual sites in a matrix starting 2 to 3 mm lateral to the animal midplane. The exposure matrix consisted of 5 laterally spaced columns and 8 rows in the vertical direction. This matrix was centered over the right uterine horn in a much more concentrated fashion than used previously. Although the restricted matrix undoubtedly did not cover the entire uterine horn, the closer spacing of individual irradiation sites was hoped to be more stressful to the fetal region than the more widely spaced matrix. The cw regime involved 100 sec. of irradiation at each of the 40 sites (5 x 8 matrix) with an intensity of 12 W cm<sup>-2</sup> SPTA. The pulse regime involved 100 sec. of irradiation at each of the 40 sites with a SPTA intensity of 450 W cm<sup>-2</sup>. The pulses were 27  $\mu$ s in duration, and delivered at a pulse repetition frequency of 1 kHz.

Animals were sacrificed by ether inhalation on day 18 of gestation and the fetuses delivered by cesarean section.

Comparisons between the right and left side for each group were performed for the number of fetuses, the number of early resorptions, the number of late resorptions, and the number of defects using signed rank tests. Defects scored were: runts, litter weight, and average weight of individual fetuses.

One-way analysis of variance was used to compare the three treatment groups (cw, pulsed, and sham) for all of the major outcome variables. When a significant difference was detected, Tukey's procedure was used to determine which of the groups differed. The fourteen outcome variables considered were: number of fetuses on the right side, left side and total; number of early resorptions on the right, left and total; number of late resorptions on the right, left and total; number of defects on the right, left and total; total litter weight; and average weight in the litter.

The results of this study are summarized in Table 1 where the means and standard deviations for each variable and group can be found. In the right and left side comparisons, only one of the twelve comparisons (three groups by four variables) was statistically significant at the .05 level. There were significantly more late resorptions on the right side than on the left side in Group A (p=.022). In addition, four comparisons were of borderline statistical significance. In Group B there were more late resorptions (p=.094) and defects (p=.060) on the right side. In Group C, the right side had more early resorptions (p=.089) while the left side had more fetuses (p=.089).

Comparison between the three groups shows that significant differences were detected for early resorptions on the left side ( $p=.002$ ), total early resorptions ( $p=.004$ ), and late resorptions on the right side ( $p=.036$ ). For both the total and left side early resorptions, Group A had significantly higher values than Groups B or C, which did not differ from each other. For the right side late resorptions, Group A is higher than Group C but Group B is not significantly different from the other two groups. Overall, any difference among the groups appears to be in the number of resorptions and not in any of the other parameters.

Maternal mortality was 43% for the pulse mode, 19% for the cw mode and 7% for sham. The more spatially concentrated irradiation matrix tended to increase maternal mortality over that seen in the previous study [Fry, 1986] where mortalities were approximately 12% for pulsed, and 10% for cw irradiations of similar ultrasound irradiation conditions. It was previously observed that maternal mortality is critically dependent on temperature [Fry, 1986]. In addition, it was previously shown that temperature rise for a pulse regime of specific temporal average intensity (like that used in this study) is substantially twice that for the same cw intensity regime. The higher maternal mortality rates in the present study are most probably associated with thermal effects.

Table 1

Variable	Pulsed irradiation	Continuous irradiation	Sham irradiation
	Group A 30 Mean (SD)	Group B 30 Mean (SD)	Group C 29 Mean (SD)
<b>Fetuses</b>			
Right	3.7 (2.0)	4.6 (2.1)	4.0 (1.8)
Left	3.9 (2.1)	4.5 (1.9)	4.8 (1.9)
Total	7.6 (3.6)	9.2 (3.2)	8.8 (2.9)
<b>Early Resorptions</b>			
Right	1.8 (1.8)	1.0 (1.3)	1.2 (1.2)
Left	1.6 (1.4)	0.8 (0.9)	0.7 (0.9)
Total	3.5 (2.9)	1.8 (1.6)	1.9 (1.4)
<b>Late Resorptions</b>			
Right	0.6 (0.9)	0.4 (0.7)	0.1 (0.4)
Left	0.2 (0.4)	0.2 (0.4)	0.1 (0.6)
Total	0.8 (1.1)	0.6 (1.0)	0.3 (0.6)
<b>Defects</b>			
Right	0.3 (0.4)	0.5 (0.8)	0.3 (0.7)
Left	0.2 (0.4)	0.1 (0.3)	0.3 (0.5)
Total	0.5 (0.6)	0.6 (0.9)	0.6 (0.8)
Litter Weight (gm)	7.46 (4.07)	8.54 (3.56)	8.23 (3.02)
Pup Average Weight	0.98 (0.17)	0.94 (0.22)	0.95 (0.21)

The results of this study on the pregnant mouse cannot be directly compared to previous studies; however, the study does appear to support the hypothesis that there are not dramatic differences in fetal effects between a high intensity pulse regime and a cw regime of the same average intensity for these severe irradiation conditions. The results of this bioeffects study are still at variance with most other published reports of pulse mode vs. cw mode irradiations. Other studies generally show the pulse mode as being a much more severe stress on tissues than cw regimes of the same average intensity.

Yours truly,

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Acknowledgments

Work supported in part by PHS Grant Number CM 30593 and the Indianapolis Center for Advanced Research, Inc.

Thanks are accorded to Eric Trauner who conducted the mouse irradiations, to Dr. Barry Katz who performed the statistical analysis, and to Clarence Reilly for assistance in aspects of data gathering and summaries.

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