

Increased Resorptions in CBA Mice Exposed to Low-Frequency Magnetic Fields: An Attempt to Replicate Earlier Observations

Jukka Juutilainen,^{1*} Hannele Huuskonen,² and Hannu Komulainen²

¹*Department of Environmental Sciences, University of Kuopio, Kuopio, Finland*

²*National Public Health Institute, Division of Environmental Health, Laboratory of Toxicology, Kuopio, Finland*

This paper has two aims. First, it reports the findings of a study on the effects of low-frequency magnetic fields on reproduction. Second, it serves as an example of an attempt to replicate the results of an experimental study in an independent laboratory and discusses some of the problems of replication studies. To try to replicate the findings of a study reporting increased resorptions (fetal loss) in mice exposed to 20 kHz magnetic fields with sawtooth waveform and to study the possible effects of 50 Hz sinusoidal fields, pregnant mice were exposed to magnetic fields from day 0 to 18 of pregnancy, 24 h per day. The flux densities of the vertical magnetic fields were 15 μT (peak-to-peak) at 20 kHz and 13 or 130 μT (root mean square) at 50 Hz. Two strains of animals were used: CBA/S mice imported from the laboratory reporting the original observations, and a closely related strain CBA/Ca. The CBA/S mice were cleaned of pathogenic microbes and parasites before they were imported into our laboratory. The magnetic field exposures did not affect resorption rate in CBA/Ca mice. In CBA/S, the frequency of resorptions was higher in the exposed mice than in the control group. However, the increase was not significantly different from either the no-effect hypothesis or the results of the original study we were attempting to replicate. Differences between the two studies and difficulties in interpreting the results are discussed. It is concluded that the results tend more to support than argue against increased resorptions in CBA/S mice exposed to the 20 kHz magnetic field. The results demonstrate that animal strain is an important variable in bioelectromagnetics research: even closely related strains may show different responses to magnetic field exposure. *Bioelectromagnetics* 18:410–417, 1997. © 1997 Wiley-Liss, Inc.

Key words: reproduction; ELF; VLF; magnetic fields; replication studies

INTRODUCTION

It is important that the results of scientific investigations are confirmed by replication in independent laboratories before they are used as a basis for making important decisions such as, e.g., exposure limits for environmental agents. In the field of bioelectromagnetics, many replication attempts have been unsuccessful. One of the reasons for this difficulty in replicating earlier findings may be that all electromagnetic field exposure parameters have not always been duplicated [Valberg, 1995]. It is also possible that, due to the complexity of biological organisms and our limited understanding of the interactions between electromagnetic fields and biological matter, researchers are not aware of all variables that should be controlled in replication attempts.

This paper has two objectives. First, it reports the findings of a study on the effects of low-frequency magnetic fields on reproduction. Second, it serves as an example of an attempt to replicate the results of an experimental study in an independent laboratory and discusses some of the problems of replication studies.

Low-frequency magnetic fields (LFMF) have been reported to affect the development of chick embryos [Juutilainen et al., 1986, 1987; Berman et al.,

Contract Grant sponsor: Swedish Work Environment Fund.

*Correspondence to: Dr. Jukka Juutilainen, Department of Environmental Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland. E-mail: juutilainen@uku.fi

Received for review 10 June 1996; final revision received 1 January 1997

1990], and the results of some epidemiological studies suggest that residential or occupational exposure to LFMFs might affect pregnancy in humans [Juutilainen et al., 1993; Lindbohm et al., 1992]. There are conflicting reports, however, and most animal studies on mammals report no or only slight effects on pregnancy [Juutilainen, 1991]. One study, however, reported robust effects on fetal development. In a study carried out in Uppsala, Sweden, increased resorptions (fetal loss) were observed in CBA mice exposed to 20 kHz, 15 μ T (peak-to-peak) magnetic fields with sawtooth waveform [Frölen et al., 1993]. The increase of resorptions was statistically highly significant, and was repeated in several experiments using a large number of animals.

The variable results obtained by different research groups may be partly explained by the different animal strains used in the experiments. Only Frölen and co-workers have used CBA mice in studying possible reproductive effects of LFMFs.

The objectives of this study were to try to replicate the results reported by Frölen et al., using 20 kHz fields with sawtooth waveform, and to study the possible effects of sinusoidal 50 Hz fields. Use of 20 kHz fields in Frölen's experiments was motivated by the presence of such fields around video display terminals, whereas exposure to 50 Hz fields is common in a wide variety of occupational and residential environments.

The original plan was to use animals imported from Frölen's laboratory. Only SPF (Specific Pathogen Free) animals are, however, allowed in the National Laboratory Animal Center of the University of Kuopio. The animals from Uppsala were found to be positive for MHV (mouse hepatitis virus) and infected by *Pasteurella pneumotrophica* and some parasites. We therefore decided to start the study using SPF-quality animals of the closely related strain CBA/Ca. Both strains originate from the same CBA/Ca strain, but the animals in Uppsala have been maintained (inbred) in Sweden for more than 60 generations and are now called CBA/S. Simultaneously, we started a process to produce SPF-quality CBA/S animals to be able to conduct additional experiments with this strain, in case we would not find any effects on CBA/Ca.

MATERIALS AND METHODS

Experimental Design

The study consisted of six experiments, with 18–60 mated females per group (Table 1). The three first experiments were identical, and the results from them were combined. The two last experiments with the CBA/S strain were conducted because the results from

the first experiments indicated that additional experiments with this strain were necessary. Because of limitations in funding, we were able to use only 20-kHz fields in the last two experiments. Group sizes were determined by power calculations [Armitage 1974]. Based on the resorption frequencies and deviations estimated from Frölen's preliminary results available when starting these experiments, 40 pregnant females per group should give a power of more than 0.8 to detect an increase in resorptions at the level of $P < .05$ (one-sided).

Animals

Mice of the strains CBA/Ca (B&K Universal Ltd., North Humperside, England) and CBA/S (The Swedish University of Agriculture, Uppsala) were used. To get SPF-quality CBA/S mice, animals from Uppsala were cleaned in Sweden (Karolinska Institute, Novum, Huddinge). Females were superovulated and mated. The embryos were transferred to healthy pseudopregnant (CBA/B6) F1 females under isolator conditions. Five animals were then imported to Kuopio to start breeding, after being checked to be free of pathogens.

CBA mice originated from a mating of Bagg albino females and DBA males at Leonell C. Strong Research Foundation, California in 1920. In 1932, the strain was imported to Great Britain, where it was used by the Royal Cancer Hospital and British Empire Cancer Campaign (CBA/Ca). The strain was brought to Harwell in 1954 (CBA/Ca;H). The CBA/Ca;H strain was brought to the Department of Genetics, University of Stockholm in 1956, and has since then been maintained (inbred) in Sweden (CBA/S). Since August 1992, SPF-quality CBA/S been maintained, first in an isolator at the University of Kuopio, and then inside the barrier at the National Public Health Institute, Kuopio, Finland.

Magnetic Field Exposure

The exposure system was different from that used by Frölen et al. [1993], but both systems produced vertical magnetic fields. The system has been used previously for exposing rats and has been described in detail elsewhere [Huuskonen et al., 1993]. It consisted of four 10-turn rectangular coils (0.425×1.205 m) in a wooden rack. Four animal cages could be placed in a rack. With six mice per cage, each rack allowed simultaneous exposure of 24 animals. The animals were exposed continuously, 24 h/day.

Part of the animals were exposed to a 20 kHz magnetic field with a triangular waveform similar to that used in the earlier experiments by Frölen et al.

TABLE 1. Description of the Experiments

Experiment	Strain	Exposure groups	Number of litters/ pregnant females	Room no.	Geomagnetic field, μT
1–3	CBA/Ca	Control	45/64	1122	34–40
		50 Hz, 10 A/m	56/66		30–34
		20 kHz	56/65		37–40
4	CBA/Ca	Control	34/35	146	45–50
		50 Hz, 100 A/m	33/35		50–55
5	CBA/S	Control	46/60	146	45–50
		20 kHz	46/59		50–55
6	CBA/S	Control	34/42	146	50–55
		20 kHz	40/43		45–50

[1993]. The peak-to-peak (p-p) amplitude of the flux density was 15 μT , with 45-s risetime and 5-s falltime. Other animals were exposed to 50 Hz fields with sinusoidal waveform. The nominal root-mean-square (rms) magnetic field strengths were 10 or 100 A/m, respectively, corresponding to flux densities of about 13 or 130 μT . Expressed as p-p values the flux densities were 36 or 360 μT . The calculated magnetic field in the animal cages varied between 94 and 117% of the nominal value. The field strength was confirmed with a calibrated coil and oscilloscope. The metal wire lid of the cages did not affect the 50 Hz field, but reduced the strength of the 20 kHz field. The current of the 20 kHz system was adjusted so that 15 μT was reached at a distance of 100 mm below the lid, the position of the animals when they stand on the surface of the bedding material. The field strength immediately below the lid was 10 μT .

The current for the 20 kHz field was produced by a function generator and an amplifier. The output signal from the generator had a rectangular waveform. However, because of the inductance of the coils, the current flowing in the coils had a sawtooth waveform. The 50 Hz current was taken from the 220 V network via an adjustable transformer. The currents were monitored continuously by a voltmeter measuring the voltage over a resistance (5.8 Ω for the 50 Hz system and 2.7 Ω for the 20 kHz system) coupled in series with the coils. The waveform was controlled by oscilloscope in the beginning and at the end of each of the six experiments and, in addition, whenever any change was observed in the voltmeter reading.

Due to a measurement error made in the beginning of experiment 6, the magnetic flux density was only 13.5 μT in the beginning of the experiment. Twenty-one of the 40 exposed animals entered the study before the error was detected, and they were thus exposed to a lower than planned flux density during at least part of their pregnancy. No difference was ob-

served in the resorption frequency between these animals and the rest of the group.

Three identical exposure racks were used, one for the 50 Hz field, one for 20 kHz, and one for sham-exposing the control animals. The distance between the two active exposure systems was 2 m, and the control animals were placed at least 3.5 m away from the closest active system to reach a flux density less than 0.1 μT .

The static (geomagnetic) field flux densities were measured and are reported here for the two rooms used in the experiments (Table 1). Inclination of the geomagnetic field varied between 60 and 73 degrees in room 1122, and from 70 to 73 degrees in room 146.

Protocol

The females were 10–13 weeks old and weighed 18–21 g at the time of mating. The males were at least 2 weeks older and had demonstrated fertility. Before mating, the animals were kept in groups of five to six males or females per cage.

The CBA/Ca mice were mated for 2–3 h before the beginning of the light period, three females per male. The CBA/S animals were mated overnight with one or two females per male. The females were examined for vaginal plugs in the morning and, if pregnant, the day was defined as day 0 of pregnancy.

Pregnant females were randomized to exposed and control groups using the stratified body weight procedure. The animals were identified by ear puncture. A single animal room was used, but each group was placed in its own cubicle. Transparent plastic cages (Macrolon, type III) were used, with aspen chips (Tapvei Oy, Kaavi, Finland) as bedding material. A maximum of six pregnant females were housed per cage. The magnetic field exposure was started in the morning after mating.

The temperature in the animal room was 22 ± 1 °C, and the relative humidity was $50 \pm 10\%$. The

lights were on 12 h per day (0700–1900 hours), and a dim “pilot” light was used during the dark period. Commercial pellets for rats and mice (R3, Astra-Ewos, Södertälje, Sweden) and tap water were available *ad libitum*. During the exposure, the animals were observed daily for clinical signs. The body weights were measured on days 0, 6, 13, and 18 of pregnancy.

The dams were killed on the day 18 of pregnancy by CO₂. The uterus and the ovaries were examined immediately for numbers of corpora lutea, implantation sites, live fetuses, dead fetuses, and resorptions. The fetuses were examined for external malformations and sex. The weight of uterus with its contents, placental weight, and weight and length of individual fetuses were measured. The empty uterus was stained by ammonium sulfide for detection of very early resorptions [Salewski 1964].

The fetuses and dams were examined using standard teratological techniques. This paper focuses on the attempt to replicate the observation of increased resorptions, and the detailed examination of fetuses and dams will be described in a separate paper.

The resorptions were classified as follows: R0 = resorption that could be detected only after ammonium sulfide staining; R1 = a small placental remnant; R2 = placental remnant with fetal membranes ≤ 3 mm; R3 = placental remnant with fetal membranes 3–5 mm; R4 = placental remnant with fetus ≤ 5 mm (corresponds to Carnegie stage 14 or less, or day 10.5 of pregnancy); R5 = placental remnant with fetus > 5 mm (stage 19, or day 13); R6 = placental remnant with fetus > 10 mm, partial destruction of tissues (fetuses with no destruction of tissues were classified as dead embryos). In addition, each resorption was described by size and color.

In experiment 2, eight females were excluded because of a leak from a water bottle during early pregnancy and one because of incorrect necropsy date. The females with the leaking water bottle had total resorptions (loss of all fetuses) or were not pregnant. Eleven females had total resorptions due to unknown reasons, and one had resorption of all but one fetus (control, experiment 5). Examination of the distribution of resorptions revealed that these cases were “outliers:” they did not fit the Poisson distribution that the other resorptions seemed to follow. These cases were not found to be related to magnetic field exposure and were thus probably due to randomly occurring maternal effects irrelevant to the purpose of this study, such as sensitivity to noise from construction work going on in another part of the building. Consequently, they were excluded from the data. Five of the excluded total resorptions were in the control groups (four in experi-

ments 1–3, one in experiments 5 and 6), three in the 20-kHz groups of experiments 1–3, and three in the 50-Hz groups (one in experiments 1–3, two in experiment 4).

For statistical analysis, three different indices were calculated to describe the number of resorptions in each exposure group. The proportion of resorptions was calculated by dividing the total number of resorptions by the total number of implantations. The proportion of litters with resorptions was calculated as the number of litters with resorptions divided by the total number of litters. The average number of resorptions per litter was calculated as the arithmetic mean (with its standard error) of the numbers of resorptions observed in individual litters. The “per litter” analysis is recommended as the best method of analysing teratological data [e.g. Haseman and Hogan, 1976], but the other two indices were calculated to facilitate comparison with the results of Frölen et al. [1993]. Only the proportion of resorptions and the proportion of litters with resorptions were reported in their study.

RESULTS

No differences were observed between the exposed and control groups in the experiments with the CBA/Ca strain (Table 2). The resorption frequency (resorptions/implantations) in the control groups was higher compared with the frequencies 5.6, 6.0, 7.2, 4.8, and 9.4% observed in the five Swedish experiments [Frölen et al., 1993].

The results from the experiments with CBA/S showed slightly increased resorptions in the exposed groups (Table 3). The difference did not reach statistical significance, but it was constant over the two experiments: the proportion of resorptions/implantations, proportion of litters with resorptions, and average number of resorptions per litter were all higher in the exposed groups in both experiments. Despite the identical animal strain, the resorption frequency of the control group was again clearly higher than in Frölen’s study. The strains CBA/Ca and CBA/S seem to have similar spontaneous resorption frequencies in our laboratory.

To study the possible effects of unknown environmental factors (e.g., temporary noise or vibration not noticed in our daytime observations), the locations of the exposed and control groups were interchanged between experiments 5 and 6. No differences were seen between the results of the two experiments.

The classified resorption data indicate that the difference between the exposed and control groups is mainly explained by the higher level of relatively early (R1) resorptions in the exposed animals (Table 4).

TABLE 2. Resorptions in CBA/Ca Mice Exposed to 50 Hz or 20 kHz Magnetic Fields

	Control	50 Hz (13 μT)	20 kHz
Experiments 1–3			
Litters	41	55	53
Implantations	347	472	455
Resorptions	39 (11.2%)	47 (10.0%)	48 (10.5%)
Litters with resorptions	24 (58.5%)	31 (57.4%)	32 (60.4%)
Resorptions/litter (±SE)	0.95 ± 0.16	0.87 ± 0.13	0.91 ± 0.13
Experiment 4			
	Control	50 Hz (130 μT)	
Litters	34	31	
Implantations	321	287	
Resorptions	29 (9.0%)	22 (7.7%)	
Litters with resorptions	20 (58.8%)	14 (45.2%)	
Resorptions/litter	0.85 ± 0.2	0.71 ± 0.2	

TABLE 3. Resorptions in CBA/S Mice Exposed to 20 kHz Magnetic Fields

	Control	Exposed
Experiment 5		
Litters	42	44
Implantations	333	358
Resorptions	38 (11.4%)	50 (14.0%)
Litters with resorptions	26 (62%)	30 (68%)
Resorptions/litter (±SE)	0.91 ± 0.14	1.14 ± 0.15
Experiment 6		
Litters	33	40
Implantations	244	271
Resorptions	30 (12.3%)	42 (15.5%)
Litters with resorptions	19 (58%)	25 (63%)
Resorptions/litter	0.91 ± 0.17	1.08 ± 0.16
Total 5 + 6		
Litters	75	84
Implantations	577	629
Resorptions	68 (11.8%)	92 (14.6%)
Litters with resorptions	45 (60%)	55 (66%)
Resorptions/litter	0.91 ± 0.10	1.11 ± 0.11

TABLE 4. Classified Resorption Data of CBA/S Mice Exposed to 20 kHz Magnetic Fields (Experiments 5 + 6)

Resorption class	Resorptions/litter ± SE	
	Control	Exposed
R0	0.013 ± 0.013	0.0
R1	0.653 ± 0.092	0.905 ± 0.104
R2	0.067 ± 0.029	0.024 ± 0.017
R3	0.040 ± 0.023	0.095 ± 0.036
R4	0.013 ± 0.013	0.024 ± 0.017
R5	0.067 ± 0.029	0.036 ± 0.020
R6	0.053 ± 0.026	0.012 ± 0.012
All resorptions	0.91 ± 0.10	1.11 ± 0.11

different way compared with our methods. According to their classification, embryos with detectable eyes were “late fetal deaths.” Removing R0, R5, and R6 from our data, gives resorption frequencies of 10.1% in the control group and 14.1% in the exposed group. These methodological differences in the evaluation of resorptions can thus only partly explain the different resorption levels observed in our study and Frölen’s.

It is worth noting that the Swedish investigators observed an exceptionally high spontaneous resorption frequency in their experiment 5, in which no difference between the exposed and control groups was observed. The control group value was 9.4%, comparable to the exposed group values found in their other experiments and close to the spontaneous resorption frequencies found in our experiments. In their experiment 5, the Swedish researchers started the magnetic field exposure on day 7. They interpreted their finding of no difference between the exposed and control groups as suggesting that early pregnancy (before day 7) is the critical period for the magnetic field effects. Another interpretation is possible: an increased resorption fre-

DISCUSSION

The reason for the high level of spontaneous resorptions in our laboratory is not known. Methodological differences could be a possible explanation for the lower resorption frequency in Frölen’s results. It is possible that they did not detect all resorptions because no staining to detect early resorptions was reported in their article. If the earliest resorptions (R0) are removed from our CBA/S data, the resorption frequency of the control group is slightly reduced (Table 4). Frölen and his coworkers also defined the difference between late fetal deaths and resorptions (early deaths) in a slightly

quency might be a very sensitive indicator of weak environmental influences, and the subtle effects of the magnetic field exposure might be observable only when the spontaneous resorption frequency is very low, i.e., when the level of other weak environmental factors is low.

The latter interpretation is supported by the results of Svedenstål and Johanson [1995]. They exposed CBA/S females to 20 kHz magnetic fields (15 μ T p-p, 40-s risetime, and 10-s falltime) for the 5.5 or 7 first days of pregnancy. In these experiments, the resorption frequency was 10.4 or 9.6% in the control groups and was not significantly altered by the magnetic field exposure. The combined data of all experiments conducted by the Uppsala group [Frölen et al., 1993; Svedenstål and Johanson, 1995] suggest that varying resorption rate in the control group has been the main difference between the positive and negative experiments. The resorption rate has been fairly constant in the exposed group, but the control group values have been low in those experiments showing a significant difference between the exposed and control groups.

The presence of infections and parasites in Frölen's animals is the only known difference between the animals used in the two laboratories. Whether this difference could have affected the resorption frequency is an interesting question. Infections and parasites affect the status of the immune system, and there are immunological reactions also between the mother and the embryo. We are not aware of any experimental studies suggesting that pathogen-free animals would have altered resorption frequencies.

If a replication study fails to repeat a statistically significant effect similar to the result of the original study, there are two possible reasons for the failure: (1) There are no effects (unsuccessful replication). This may be due to a false-positive finding in the original study or to methodological or other differences between the two studies. (2) There is an effect, but the effect is weaker in the replication study than in the original study. This may be due to chance or differences between studies.

No magnetic field effects on resorptions were observed in the experiments with CBA/Ca mice, so those experiments clearly belong to the first category. Different animal strain is one of the possible explanations for the failure to replicate Frölen's results. The interpretation of the CBA/S results is more difficult. It is important to analyze all possible differences between laboratories that may have affected the results. In addition to the methodological difference in observing the resorptions, there were differences between our and Frölen's laboratories in the exposure systems, ambient

geomagnetic fields, and the microbiological/parasitological status of the animals.

The exposure system used by Frölen and his co-workers consisted of circular coils forming a solenoid-like structure, whereas we used rectangular coils. Both systems, however, produced vertical magnetic fields. In our study, the metal wire lids of the plastic animal cages attenuated the 20 kHz fields close to the lid. Similar plastic cages were used in Frölen's study, but the lids were modified by insulating the wires to avoid loop-like conducting structures and to block the induced eddy currents responsible for field attenuation. The field intensities were, however, identical in the area where the animals spend most of the time, so it is unlikely that the weaker fields close to the lid have affected the results of our study. The different inductance-resistance combinations of the two exposure systems may have caused differences in the waveforms. Both groups report similar risetimes and falltimes of the sawtooth waveform, but minor differences in the waveforms cannot be excluded. Without mechanistic understanding of the possible magnetic field bioeffects, it is not possible to estimate the significance of such small waveform differences. Magnetic field exposure systems may produce non-EMF exposures such as mechanical vibrations or radiant heat that could affect the exposed animals. Because of the low currents used, radiant heat was negligible in both laboratories. The level of mechanical vibration was not reported in Frölen's study, but the structure of the exposure system suggests that the vibration level was probably very low.

The static (geomagnetic) fields in Frölen's study varied between 39 and 46 μ T and had a major vertical component. Differences in the static magnetic field may be important for the biological effects if the effects of alternating magnetic fields are based on resonance-type interaction mechanisms requiring given combinations of static and alternating magnetic fields [Liboff et al., 1990, Blanchard and Blackman 1994]. These hypothetical interaction mechanisms, however, have not been generally accepted, and the geomagnetic field differences between our and Frölen's laboratories were smaller than the within-laboratory variations.

As discussed above, differences in the microbiological/parasitological status of the animals may be an explanation for the different levels of spontaneous resorptions observed in the two laboratories. If so, it may also have influenced the magnetic field sensitivity of the animals. Again, this is only speculation; there is no previous evidence of such influences.

If we assume that all the above-mentioned differences between laboratories are unimportant, there is one more possible explanation for the different results

of the two studies: chance. To reduce the effect of chance, replication studies should be designed to have a high enough statistical power to detect an effect of the size reported in the original study. Too small studies produce no information, and should not be used as evidence for or against the original study. The present study was originally planned to have a power of at least 0.8 to detect an effect (at $P < .05$) equal to that reported in Frölen's study. The combined analysis of experiments 5 and 6 should have had a much higher power. However, the power calculations made before the experiments (as they always are) were based on expected group differences and standard deviations. As usually, the observed values were different from the expected ones. In this case, the higher than expected standard deviation (associated with the high resorption frequency in the control group) reduced the probability of finding a statistically significant effect.

In replication studies, it is not enough to use the standard way of evaluating the importance of chance by testing whether the result is statistically significantly different from the no-effect hypothesis. One should also ask whether the result is different from the alternative hypothesis of effects equal to those observed in the original study. We already know that the baseline frequency of resorptions in the control group (R_c) is, for unknown reasons, higher in our than in Frölen's laboratory. If we assume that the increase caused by magnetic fields is additive, the difference $\Delta R = R_e - R_c$ (where R_e is the resorption frequency of the exposed group) can be used for comparing the results of the two studies. In our experiments ΔR was $14.6\% - 11.8\% = 2.8\%$ (standard deviation 1.95%), a value not significantly different from zero ($P = .075$). An ΔR of 4.8% was observed in Frölen's study (combined data of experiments 1–4, values from 3.7 to 6% were found in individual experiments). Our result was not significantly different either from zero or from Frölen's data. The ΔR value given by our study, however, differed more from zero than from the ΔR found in Frölen's study, suggesting that our data are more consistent with their data than the no-effect hypothesis. A similar comparison can be made also for the resorptions per litter data. In our study, the difference ($1.11 - 0.91 = 0.20$) was not statistically significant, but it differed slightly more from zero than from Frölen's results (combined data of experiments 1–4: $0.79 - 0.31 = 0.38$). The third resorption index, proportion of litters with resorptions, was very high in the control group in our study, and the difference between the exposed and control groups was only 6% ($66\% - 60\%$). This finding is closer to zero than to the difference of 20% ($53\% - 33\%$) found in Frölen's study.

CONCLUSIONS

The results suggest that the 50 Hz or 20 kHz magnetic fields used in this study do not induce resorptions in CBA/Ca mice. The results from the experiments with CBA/S are more difficult to interpret. However, together with the earlier observations of Frölen et al. [1993], they can be considered to be more for than against increased resorptions in CBA/S mice exposed to a 20 kHz, 15 μ T (p-p) magnetic field with sawtooth waveform and risetimes and falltimes of 45 s and 5 s, respectively. The result gives limited support to the hypothesis that low-frequency magnetic fields might in some conditions affect embryonal development in mammals. Additional experiments are recommended to confirm the findings and to investigate whether 50 Hz fields have similar effects. The results demonstrate that animal strain is an important variable in bioelectromagnetics research: even closely related strains may show different responses to magnetic field exposure.

ACKNOWLEDGMENTS

We are grateful to Dr. Britt-Marie Svedenstål for kindly providing information about the details of the original study we were attempting to replicate. We also wish to express our warmest thanks to Lars Ährlund-Richter for cleaning the CBA/S mice to produce SPF-quality animals.

REFERENCES

- Armitage P (1974): "Statistical Methods in Medical Research." Oxford: Blackwell Scientific Publications.
- Berman E, Chacon L, House D, Koch BA, Koch WE, Leal J, Løvtrup S, Mantiply E, Martin AH, Martucci GI, Mild KH, Monahan JC, Sandström M, Shamsaifar K, Tell R, Trillo MA, Ubeda A, Wagner P (1990): Development of chicken embryos in a pulsed magnetic field. *Bioelectromagnetics* 11:169–187.
- Blanchard JP, Blackman CF (1994): Clarification and application of an ion parametric resonance model for magnetic field interaction with biological systems. *Bioelectromagnetics* 12:217–238.
- Frölen H, Svedenstål B-M, Paulsson L-E (1993): Effects of pulsed magnetic fields on the developing mouse embryo. *Bioelectromagnetics* 14:197–204.
- Haseman JK, Hogan MD (1976): Selection of the experimental unit in teratology studies. *Teratology* 12:165–172.
- Huuskonen H, Juutilainen J, Komulainen H (1993): Effects of low frequency magnetic fields on fetal development in rats. *Bioelectromagnetics* 14:205–213.
- Juutilainen J, Harri M, Saali K, Lahtinen T (1986): Effects of 100-Hz

- magnetic fields with various waveforms on the development of chick embryos. *Radiat Environ Biophys* 25:65–74.
- Juutilainen J, Läärä E, Saali K (1987): Relationship between field strength and abnormal development in chick embryos exposed to 50 Hz magnetic fields. *Int J Radiat Biol* 52:787–793.
- Juutilainen J (1991): Effects of low frequency magnetic fields on embryonic development and pregnancy. *Scand J Work Environ Health* 17:149–158.
- Juutilainen J, Matilainen P, Saarikoski S, Läärä E, Suonio S (1993): Early pregnancy loss and exposure to 50-Hz magnetic fields. *Bioelectromagnetics* 14:229–236.
- Liboff AR, McLeod BR, Smith SD (1990): Ion cyclotron resonance effects of ELF fields in biological systems. In Wilson BW, Stevens RG, Anderson LE (eds): “Extremely Low Frequency Electromagnetic Fields: The Question of Cancer.” Richland, WA: Battelle Press, pp 251–289.
- Lindbohm M-L, Hietanen M, Kyyrönen P, Sallmén M, von Nandelstadh P, Taskinen H, Pekkarinen M, Ylikoski M, Hemminki K (1992): Magnetic fields of video display terminals and spontaneous abortion. *Am J Epidemiol* 136:1041–1051.
- Salewski E (1964): Färbemethoden zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* 247:367.
- Svedenstål B-M, Johansson K-J (1995): Fetal loss in mice exposed to magnetic fields during early pregnancy. *Bioelectromagnetics* 16:284–289.
- Valberg PA (1995): Designing EMF experiments: What is required to characterize “exposure?” *Bioelectromagnetics* 16:396–401.