

Effects of Long-Term Exposure to a 400-kV, 50-Hz Transmission Line on Estrous and Fertility in Cows

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ABSTRACT

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During recent years, concerns about high-voltage transmission lines have included possible direct influence on fertility of livestock reared near them. The objective of this study was to investigate, by an experimental design, the influence on reproductive performance in cows of a continuous long-term exposure to high-voltage AC transmission lines.

The study comprised one exposed and one control group of 58 Swedish Red and White breed heifers each. Exposed animals were kept in three 44-m-wide enclosures beneath a 400-kV, 50-Hz transmission line, while control animals were kept in three similar enclosures off the line, each approximately 0.7 ha. The animals were put into the enclosures in the beginning of June and the inseminations started 24 days thereafter. The exposure time was, on an average, 120 days continuously.

In the exposed group, the average exposure to the unperturbed electric field was measured and calculated to be 4 kV/m and that of the magnetic field to be 2 μ T.

Estrous was detected by systematic observations. Inseminations were carried out according to ordinary AI regimes by the local AI centre, with semen from one single ejaculate.

The parameters studied expressed length and regularity of the estrous cycle, mid-cycle plasma progesterone level, intensity of estrous, number of inseminations per pregnancy and proportion of animals conceiving. Also the proportion of fetuses showing heart activity shortly after slaughter of the dam was studied. None of the parameters were found to be influenced by the exposure of the heifers to the 400-kV, 50-Hz transmission line.

INTRODUCTION

High-voltage transmission lines are known to emit electric (E-) and magnetic (B-) fields, noise and air ions. Electromagnetic fields induce currents in the body of an animal or person in the field (Kaune and Phillips, 1980; Kaune and Gillis, 1981). Biological effects resulting from exposure to electric and

magnetic fields have been demonstrated in vertebrates, as concluded in several reviews (Waskaas, 1981; Phillips, 1982; Algers and Hennichs, 1983; Sheppard, 1983).

Free et al. (1981) found a change in the cyclic variations of follicle-stimulating hormone and corticosterone in weanling rats exposed to 80-kV/m (unperturbed intensity), 60-Hz electric field. The authors concluded that this change may be related to alterations in episodic rhythms.

Effects of electromagnetic fields on fertility in both animals and man have often been discussed. Diverging results have been reported concerning effects of pulsed magnetic fields of power frequency on embryonal development in chickens (Delgado et al., 1982; Ubeda et al., 1983; Maffeo et al., 1984). Seto et al. (1984) could not reveal any effects on fertility or malformations in four generations of rats, continuously exposed to 60-Hz, 80-kV/m (unperturbed intensity) electric fields.

During recent years, concerns about high-voltage transmission lines have included possible direct influence on livestock reared near them. Thus, farmers have attributed detrimental effects on animal reproduction to the presence of these lines (Algers and Hennichs, 1985). Very few controlled experiments have examined such effects. A 6-year screening study of miniature swine exposed to a 30-kV/m, 60-Hz electric field for 20 h/day over three generations (Anderson and Phillips, 1985) revealed an increased prevalence of morphological malformations and lower body weights in some categories, although no significant influence of electric-field exposure on reproduction was shown. The studies on swine by Mahmoud and Zimmerman (1984) and on cattle by Williams and Beiler (1979), Amstutz and Miller (1980) and Martin et al. (1983) did not give information on actual field exposure and perturbation of the electric field nor did they take shielding from roofs into account. In the latter cases, the fraction of time that the animals spent beneath the transmission lines was not recorded. In none of these studies were effects on reproduction reported.

In a Swedish pilot study (Algers et al., 1981), evaluation of artificial insemination records of 36 herds grazing beneath 400-kV, 50-Hz transmission lines during one or two summers failed to demonstrate effects on fertility. The cows in this study were exposed to the transmission lines for only 7 days per year, on average. A more extensive nationwide survey of 106 herds which were exposed to the same type of lines for > 15 days per year (Algers and Hennichs, 1985), revealed no overall reduction in fertility. However, as the Swedish 400-kV network was mainly built > 10 years before the years studied, the affected farmers would have had time to adjust to a moderate effect on fertility in the form of giving up rearing cattle, changing pastures, keeping cows indoors that did not conceive outdoors or making use of autumn calving. Also, to some extent, the herdsman could have compensated a minor disturbance in the fertility of his herd by increasing his effort in the detection of signs of estrous. It was not

possible to take these possible adjustments into account in the studies mentioned.

The Swedish high-voltage distribution network is presently designed for 50 Hz and voltage levels up to 400 kV.

An interventive study of a constant and long-term exposure of cattle to 400-kV, 50-Hz lines under practical conditions (Algers and Hultgren, 1986) was designed to detect possible effects on estrous signs or fertility. The objective of the study was to detect possible major effects on reproductive performance in cows. Effects consisting of a prolonged, shortened or irregular interovulation interval, or a deteriorated function of corpora lutea as can be seen from the mid-cycle plasma progesterone level, reduced intensity of estrous, a higher number of inseminations per pregnancy and/or fewer pregnancies and reduced fetal viability were hypothesised.

MATERIAL AND METHODS

The study comprised one exposed group and one control group, both finally containing 58 individuals. The risk factor studied was exposure to an ordinary 400-kV, 50-Hz transmission line, which implies all physical phenomena caused by the line. The exposure was accomplished from June to mid October 1985, which is referred to here as the study period. The length of the period averaged 120 days (range 94–132 days) in both groups.

Animals and their handling

Altogether 124 virgin heifers were purchased from 39 farms approximately 1 month before the start of the study period. Finally, 116 heifers were accepted according to breed (the Swedish Red and White breed), size (at purchase at least 142 cm heart girth), state of health (no serious pathological changes found upon examination of legs, hoofs, body condition, hair coat, heart sounds and blood serum and no serious pathological changes reported by the owner/vendor), sexual status (no clinically detectable pregnancies, pathological changes in cervix, uterus, oviducts and ovaries or malformations in the sexual organs) and ease of handling (coming into the stanchions when fed).

When allocated to the study groups the animals from each farm were divided evenly (± 1) between one exposed enclosure and its respective control enclosure but otherwise allocation was carried out randomly.

The mean weights at the beginning of the study period in the exposed and control groups were 340 kg (range 255–460 kg) and 344 kg (range 230–440 kg), respectively. Luteal activity was screened by taking blood samples for analyses of plasma progesterone on one to three occasions before the study period, at intervals of 6 or 7 days. The analyses were carried out by a radioim-

munoassay (Bosu et al., 1976; Castellanos, 1979). Progesterone levels >3 nmol/l were regarded as positive. Luteal activity was displayed by 44 heifers (76%) in the exposed group and 48 heifers (83%) in the control group. In 24 animals (21%) a high progesterone level was not found. These were judged as sexually immature but normal and most probably ovulating within a few weeks. Hence they were accepted.

Systematic precautions were taken against endoparasites. These included treatments with oxfendazole and ivermectin as required. Blood samples were collected from all animals to analyse the serum antibody titres for bovine virus diarrhoea virus (BVDV). These samples were analysed by a modified serum neutralization test (Castrucci et al., 1975). Serum from the seronegative heifers was further analysed for the presence of BVDV by a modified indirect immunofluorescence technique (Bielefeldt Ohmann, 1983). At the end of the study period, further analyses of BVDV antibody titres were made in animals earlier found to be seronegative. One animal was rejected due to seroconversion. Of the animals finally accepted, 31 (53%) were seropositive in both groups during the whole study period.

Two assistants were responsible for feeding, estrous detection, assistance and general care in Enclosures 1-2 and 3-6 (see below), respectively. Once every morning, at feeding time, all animals were locked up in stanchions, and examinations and treatments were made. The animals were given free access to clover/grass-hay, of which they consumed approximately 5-6 kg per individual per day, in bunks along the stanchions. Minerals and salt were added. Additionally, in order to induce the animals to enter the stanchions in Enclosures 1 and 2, about 0.2 kg rolled oats was given to each animal until the middle of September. From then on, when all inseminations were made, about 1 kg rolled oats was given to each animal in all enclosures. The animals were given access to water in two or three drinking cups in each enclosure.

All animals were slaughtered at the end of the study period.

Test sites and exposure

Two test sites (A and B) were located in the central part of southern Sweden. The animals were kept in six enclosures, Nos. 1 and 2 at Site A and Nos. 3-6 at Site B. Nos. 1, 3 and 4 were exposed to the transmission lines. Each enclosure contained 19-20 study animals. Hence, Site A had 19 exposed animals out of 39 (49%), compared to 39 exposed out of 77 (51%) at Site B. In order to minimize the influence of confounding factors, the enclosures were paired 1 + 2, 3 + 6 and 4 + 5, with respect to the allocation of animals, the enclosure conditions and various measures during the study.

The width of the enclosures was set according to the definition of exposed area used in previous Swedish publications (Algers et al., 1981; Hennichs, 1982), i.e. the area between two straight parallel lines drawn 10 m outside the

TABLE I

Estimated ranges of E- and B-field intensities at 50 Hz in the two experimental groups (measuring point means during the study period)

Group	E-field (kV/m)		B-field (μ T)	
	Min.	Max.	Min.	Max.
Exposed	1.4	8.4	0.39	4.7
Controls	0.0	0.1	0.004	0.07

outer conductors of the 400-kV line. Thus each of the exposed enclosures was 44 m wide and 0.64–0.75 ha. None of the enclosures was located in the vicinity of farm buildings, major roads or other public facilities. There were no trees, bushes, watercourses or major slopes in any of them which could result in shielding of the animals from the electromagnetic fields. The enclosures were fenced with a conventional barbed-wire fence, which was adequately grounded to eliminate capacitive tensions and reduce inductive tensions in it. At the feeding sites, sections of conventional self-locking steel stanchions were erected and adequately grounded.

On Site A the drinking cups were connected to a water-main. On Site B two wells were dug, one for the exposed group and one for the controls. The hygienic quality of the water in the wells was monitored throughout the study period.

Because of the unusually heavy rainfall during June the ground became very muddy around the stanchions and for that reason the number of animals in Enclosures 3–6 was reduced to ten in early September. The 40 animals slaughtered at that time had been pregnant for at least 47 days.

The 400-kV line used in this study was part of the ordinary power distribution network. At both study sites, 30 m away from, and parallel to, the 400-kV line's center conductors, there was a three-phase 130-kV, 50-Hz line. According to substation power recordings there were no power failures on either line during the study period.

Measurements and calculations were made to describe the mean unperturbed E- and B-field intensities at 50 Hz in different parts of the exposed and control enclosures. Voltage and current estimations were based on data from the Swedish State Power Board. For the E-field measurements a non-commercial device developed at the Institute of High Voltage Research (Lövstrand, 1976) was used and the B-field measuring device used was a flux density meter developed and constructed at the Swedish power company Sydkraft and designed for 50 Hz (Anon., 1984). The E- and B-field intensity in those measuring points having the lowest and highest mean values during the study period are shown in Table I.

Calculations were made to describe the average individual's mean exposure

to 50-Hz unperturbed electric and magnetic fields during the total 120-day period (for further details see Algers and Hultgren, 1986). These averages were 4 kV/m unperturbed E-field, 2 μ T total B-field and 1 μ T vertical B-field in the exposed group, and in the control group were 0.0 kV/m, 0.02 μ T and 0.02 μ T, respectively.

Reproductive measures

The estrous detection started on the first day of the study period, and it consisted of the following three procedures:

(1) Observations of the herd, mainly from outside the enclosures, for 1 h every morning, between 06.30 and 08.30 h, (except Sundays) and 1 h every afternoon, between 16.00 and 18.00 h. This routine was carried out simultaneously in Enclosures 1 and 2, and, by the other assistant, first in Enclosures 5 and 6 and then, approximately 1 h later, in Enclosures 3 and 4.

(2) Close examination of each individual, made every day between 08.30 and 10.00 h.

(3) The AI technician, when sent for, assessed the signs of estrous and could, according to the normal AI routine, influence the decision of whether to inseminate or not.

Each day the animals were classified on the basis of the respective symptoms recorded, as 'not in standing estrous', 'probably in standing estrous' or 'in standing estrous'. This classification was made by the respective assistant. Only heifers 'in standing estrous' were submitted to insemination. The main criterion for standing estrous was a recording of the heifer obviously allowing mounting by other animals. As the animals were not observed for all 24 h of the day, other signs, such as ruffling of the rump hair or abrasion of the rump skin, the heifer mounting other animals, spontaneous lumbar lowering and pronounced local signs of estrous, were also regarded as indicators of standing estrous, in progress or missed by a few hours. These additional signs had to be evident or pronounced and one sole sign was not accepted as a criterion for standing estrous, nor was 'uncertainly standing when mounted'. Such cases were instead denoted as 'probably in standing estrous'. Analysed plasma progesterone levels (see below) were not used for estrous detection.

Each animal's mean intensity of estrous was calculated as an average sum of weighted values of signs on the day before each day of ovulation (see below). The following signs and grades were used:

General activity	Normal	0
	Markedly increased	2
Mounting	None	0
	Occasional	1
	Repeated	2

Standing when mounted	None or uncertain	0
	Obvious	1
Lumbar lowering	None or when touched	0
	Spontaneous	1
Restlessness, bellowing	Not obvious	0
	Obvious	1
Vulvar swelling	None	0
	Mild	1
	Pronounced	2
Vulvar reddening	None	0
	Mild	1
	Pronounced	2
Quantity of clear discharge	None	0
	Mild or pronounced	2

If standing estrous was recorded in the morning (in this study 71%) insemination was carried out the same day, and if it was recorded in the afternoon (here 29%) insemination was carried out the next day. Insemination could be repeated in the same estrous if standing estrous was observed for > 1 day. In that case, however, all inseminations during a 6-day period were regarded as one insemination. All inseminations were carried out in the morning, by a total of six well experienced AI technicians from the local AI centre. All semen used was taken from one single ejaculate of a Finnish Ayrshire bull. Prior to the study the bull was preliminarily AI tested in 110 inseminations, showing a fertility above average. Inseminations were started on Day 24 of the study period. Each animal was then inseminated at the first detected standing estrous, and inseminations were made on a maximum of three estrous occasions per animal.

During the study period we collected blood samples for analyses of plasma progesterone with the intention of recording ovarian functions and detecting reproductive disorders. The samples were taken from the jugular veins at intervals of 6–8 days until the first estrous was recorded. Following an estrous one sample was taken within 2 days (preferably not on the insemination day) and again at 10–12 days after the estrous recording. All samples were analysed by a radioimmunoassay (Bosu et al., 1976; Castellanos, 1979).

The ovulation day of each cycle was determined with the help of recorded signs of estrous and progesterone levels. By definition, ovulation was judged to occur when at least one of the following three situations prevailed:

(1) A standing estrous or a suspected one, in combination with an 18–24-day interval to the previous estrous or to the following estrous or a blood discharge recorded within 5 days ahead and no high progesterone level (>4 nmol/l) recorded within the period 2 days before–2 days after the estrous. Ovulation was recorded as occurring the day after estrous recording.

(2) A blood discharge, in combination with no high progesterone level (> 4 nmol/l), within the period 5 days before–2 days after the discharge, and an estrous recorded 18–28 days back or 14–24 days ahead, or another blood discharge recorded 16–26 days ahead. Ovulation was recorded as occurring the day before the blood discharge.

(3) A low progesterone level (≤ 4 nmol/l) recorded, in combination with a high progesterone level (> 4 nmol/l) within the past 18 days and a high progesterone level (> 4 nmol/l) within 14 days ahead and in combination with a recording of estrous signs. Ovulation was recorded as occurring the day after the estrous signs.

The ovulation day was designated Cycle Day No. 0, and in the following text we refer to this numbering of the cycle days. Thus, not only cyclic but also pregnant animals were included in this definition.

During the study period, systematic clinical examinations were made to detect ovarian activity, pregnancy and sexual disorders. Rectal palpations were carried out by the same veterinarian throughout the study. They were made at 12–16-day intervals until each animal's first estrous. Following each recorded estrous an examination was made mid-cycle. Pregnancy was detected from 44 days after insemination. Furthermore, rectal palpations were carried out whenever needed for common clinical reasons.

Examinations at slaughter

All sexual organs were examined at slaughter, not earlier than at 35 days of pregnancy, to enable a rough assessment of the viability and development of the fetuses. The free passage of all oviducts was checked, as well as pregnancies and abnormalities of the uteri and ovaries. The fetal viability was recorded 45–60 min after the dams were stunned. The uteri were opened and the fetal thoraces were cut open ventrally to display the heart. The heart was observed for 30 s; one or more heart beats was denoted 'living fetus'. All fetuses were examined for gross malformations in skeleton and extremities, central nervous system, eyes and viscera. Histological samples were taken from the endometrium of six non-pregnant animals (four exposed and two controls), examined for the presence of pathological changes possibly preventing pregnancy.

Analysis of data

Analyses were accomplished by the use of multiple linear regression models (Armitage, 1971), Student's *t*-tests, z_c -tests for comparison of proportions and *F*-tests (Sokal and Rohlf, 1981). In the multiple regression models the parameters under study served as dependent variables, and high-voltage line exposure (0=control, 1=exposed) together with relevant confounding factors served as independent variables. The *t*- and z_c -tests were performed with no

regard for confounding factors. The parameters under study were (1) cycle length prior to first service, (2) cycle length post first service, (3) mid-cycle plasma progesterone level, (4) intensity of estrous, (5) number of inseminations per pregnancy, (6) conception rate at slaughter, (7) proportion of fetuses living at slaughter and (8) regularity of the cycle lengths prior to and post first service (analysed by *F*-tests on the standard deviations of these intervals in the two groups). Cycle length was defined as inter-ovulation interval. The individual was regarded as the unit of analysis. Individual means were calculated in cases of several observations per animal.

The following confounding factors were included in the multiple regression models:

Sexual maturity (plasma progesterone levels recorded before the start of the study period; 0 = luteal activity not found, 1 = activity found);

Weight (live weight in kg at the start of the study period);

Growth (daily weight gain in kg);

Infections and trauma (significant illness, e.g. respiratory diseases, keratitis, vulvar wounds, other severe wounds and severe lameness; 0 = illness not recorded, 1 = illness recorded);

AI technician 1, 2 and 3 (proportion of all inseminations, in each animal, executed by the respective AI technician; inseminations performed by Technicians 4–6, thus resulting in all these three proportions decreasing);

Insemination note (proportion of all inseminations in each animal indicated as in some way troublesome by the AI technician, e.g. 'restive', 'narrow cervix' or simply 'difficult to inseminate').

Site (test site; 0 = Site B, 1 = Site A);

The factors *AI technician 1, 2, 3* and *insemination note* were used only in those regressions concerning cycle length post first service, number of inseminations per pregnancy and conception rate. All other factors were used in all regressions.

RESULTS

Three infectious diseases were diagnosed, i.e. dermatophytosis in ~50 animals, keratoconjunctivitis in ~50 animals and a mild respiratory disease in five animals. Eleven animals had minor wounds or other traumatic injuries. None of the diseases resulted in any further complications. No hoof diseases occurred. The diseases occurred similarly in the two groups. Only 15 heifers showing keratitis had to be treated, which was done successfully with antibiotics. In one case, anestrus was diagnosed and believed to be caused by malnutrition. After repeated rectal examinations and analyses of plasma progesterone one case of persistent corpus luteum was diagnosed. This animal was treated with prostaglandin $F_{2\alpha}$, whereafter she ovulated and was inseminated.

TABLE II

Comparison of reproductive performance and fetal viability in exposed and control groups by *t*-test and *z_c*-test analyses

Parameter	Exposed			Controls			Critical ratio
	<i>x</i>	<i>s</i>	<i>n</i>	<i>x</i>	<i>s</i>	<i>n</i>	
Cycle length prior to first service (days)	21.0	1.54	39	20.9	1.68	43	0.19 ¹ n.s.
Cycle length post first service (days)	23.9	5.21	16	24.7	6.66	14	0.36 ¹ n.s.
Mid-cycle plasma progesterone level (nmol/l)	24.1	4.79	55	25.0	4.73	54	0.97 ¹ n.s.
Intensity of estrous (score)	8.72	2.74	56	9.46	2.76	55	1.41 ¹ n.s.
Inseminations per pregnancy	1.22	0.46	51	1.19	0.44	52	0.26 ¹ n.s.
Conception rate (proportion)	0.88	-	58	0.90	-	58	0.29 ² n.s.
Living fetus (proportion)	0.63	-	51	0.50	-	52	1.30 ² n.s.

¹*t*-value.

²*z_c*-value, including continuity correction.

n.s. = not significant.

The mean daily weight gain in the two groups was 0.19 kg (range -0.7-0.8 kg) and 0.24 kg (range -1.0-0.7 kg), respectively.

In total, 124 cases of standing estrous and 131 ovulations were recorded in the exposed group, and 121 cases of standing estrous and 130 ovulations in the control group. False positive cases of estrous were defined as recordings of standing estrous without any ovulation recorded within 5 days earlier or later, and false negative cases of estrous as recordings of ovulation without any standing estrous recorded within 5 days earlier or later. The numbers of false positive cases of estrous, among all standing estrous recordings, were 14 (11%) in the exposed group and 9 (7%) in the control group. The corresponding numbers of false negative cases of estrous, among all ovulation recordings, were 21 (16%) and 19 (15%), respectively.

One animal in each group was never inseminated, due to weak estrous or anestrus.

The reproductive performance in the two groups is compared in Tables II and III.

Of 112 mid-cycle progesterone samples in the exposed group, 30% were collected following ovulations at which the heifers were known to be cyclic, and

TABLE III

Comparison of reproductive performance in exposed and control groups by multiple linear regression analyses

Parameter	<i>b</i>	s.e. _{<i>b</i>}	<i>C_D</i>	<i>n</i>	<i>P</i>
Cycle length prior to first service (days)	0.08	0.38	0.04	82	0.84 n.s.
Cycle length post first service (days)	-0.22	2.24	0.48	30	0.92 n.s.
Mid-cycle plasma progesterone level (nmol/l)	-0.64	0.91	0.09	109	0.49 n.s.
Intensity of estrous (score)	-0.88	0.52	0.10	111	0.09 n.s.
Inseminations per pregnancy	0.06	0.09	0.18	102	0.54 n.s.
Conception rate (proportion)	-0.04	0.06	0.06	113	0.46 n.s.

b, regression coefficient; s.e._{*b*}, standard error of *b*; *C_D*, coefficient of determination. n.s., not significant.

49% from heifers known to be pregnant. Of 100 samples in the control group these figures were 29 and 50%, respectively.

No significant relationships between exposure to the high-voltage line and the parameters under study were found in the *t*-test, *z_c*-test or multiple regression analyses. No significant difference was found between the two groups regarding regularity of the cycle lengths prior to and post first service, ($P > 0.05$), the *F*-ratios being 1.10 and 1.28, respectively (using the standard deviation of the controls in the numerator). The regression analyses revealed significant relationships between the mid-cycle progesterone level and *site* (regression coefficient (b) = -3.07, $P < 0.01$), between intensity of estrous and *growth* (b = -2.18, $P < 0.05$) and between number of inseminations per pregnancy and *trauma* (b = 0.74, $P < 0.001$). No other significant relationships were found between the parameters under study and the risk factors included.

Cystic corpora lutea were found in five animals of which two were exposed. All these animals were non-pregnant. No other changes possibly related to pregnancy and no malformations were found at slaughter.

DISCUSSION AND CONCLUSIONS

The study was designed to detect a difference between the conception rate percentages in the two groups of more than 20, an effect suggested by two earlier herd investigations (Algers et al., 1981). Furthermore, the study period

chosen in this study was approximately two to five times longer than the corresponding exposure time per year in those two herds investigated, and 15–20 times longer than the average per year (i.e. of the total time, not necessarily continuous) in all Swedish dairy herds exposed to 400-kV lines (Algers et al., 1981). Thus, an effect found in this study probably would correspond to a much smaller effect under practical conditions. Hence, possibly existing effects of practical interest were expected to be detected, although even smaller effects might be of general biological interest.

Our intention was to reproduce typical pasture conditions for Swedish heifers or dairy cows, except for the high-voltage line exposure time. By standardizing various measures and observation procedures, and by pairing enclosures with regard to the allocation of animals and other relevant conditions, systematic errors due to e.g. estrous detection possibilities, feed and water quality, feeding regime, working schedule, climate, ground conditions, data recording and general handling and care were avoided or greatly reduced. Thus, the study could be regarded as a controlled experiment. Nevertheless, we wanted to take identified potentially confounding factors into account in the analyses, and this was done by using the multiple linear regression models described. This technique was judged to increase the power of the analyses considerably. However, when drawing conclusions from the results of the multiple regression analyses, it must be remembered that certain factors were eliminated, which may not very often be the case under natural circumstances. In other words, due to the influence of these factors, an effect of electromagnetic fields on for instance the fertility of cows may (or may not) be seen in real life. Therefore, the *t*- and *z_c*-tests were made to estimate the influence of the total experimental situation, including any effects of the confounding factors mentioned. For practical reasons the study could not be carried out completely blindly, although a blind technique was applied in all blood sample analyses and post mortem examinations.

Weight, growth during the study period and sexual maturity prior to the study period were assumed to affect the reproductive functions. According to the analyses, *weight* and *sexual maturity* were not found to be associated with any of the parameters studied. *Growth*, on the other hand, showed a significant negative association with intensity of estrous. This association, however, could not be explained and was considered to be caused by chance. The relatively poor growth of the animals was explained by the bad weather conditions during most of the study period in combination with a relatively modest energy content of the hay. It was judged that there was a higher risk of influencing fertility by changing the feeding regime during the period of inseminations than by using the original regime.

The health condition of the animals could be regarded as fairly good in all enclosures. The highly significant positive association between *trauma* and number of inseminations per pregnancy was expected and may demonstrate

the importance of avoiding painful diseases. Due to the heavy rain, the feeding areas in the enclosures became very muddy. The risk of hoof diseases occurring was considered to increase at that time and as such painful diseases might influence fertility it was decided to reduce the number of animals in four enclosures. This was done by slaughtering the animals recorded pregnant for at least 47 days, in accordance with the exposure plan.

The factor 'site' in the multiple regression models includes a great number of subfactors regarding variations between Sites A and B, e.g. ground conditions and general handling and care. Interaction between some of these subfactors cannot be ruled out and could have occurred between e.g. ability to detect estrous signs and ground conditions. Nevertheless, the factor, *site*, was judged to express the suggested subfactors satisfactorily. There does not seem to be any reasonable explanation of the significant association found between mid-cycle plasma progesterone level and *site*.

Among the risk factors used to increase the validity of the multiple regression analyses, seven factors (*growth, infections, trauma, AI technician 1, 2, 3* and *insemination note*) occurred during the study period and thus could not be controlled or distributed randomly by the time of allocation of the animals. These factors were included in the analyses, because their occurrence was considered not to be caused by the exposure situation. Two factors (*sexual maturity* and *weight*) were distributed randomly at the start of the study period. Finally, the factor *site* was included because Site A did not have the same proportion of exposed animals as in Site B.

Feed or water of a poor quality given systematically to one of the two groups might have influenced the fertility in that group (Swensson and Olsson, 1981). As the quality of the hay varied, and as exposed and control enclosures at Site B used one well each, the risk of such an effect occurring existed. However, owing to the hygienic quality of feed and water, according to analysis, and the similar situation in the two groups, feed and water were judged not to have any detrimental influence on the results.

Spark discharges between the animals and electrically charged objects were avoided as such discharges may induce chromosomal aberrations and fertility disturbances in males (Nordström et al., 1983; Nordenson et al., 1984). It is not known whether such discharges influence the fertility in cows.

The control enclosure at Site A was located only 60–95 m off the line. Still, the highest B-field in the parts nearest to the line was only $0.07 \mu\text{T}$, in comparison with $0.39 \mu\text{T}$, the lowest B-field intensity in the exposed enclosures. On average, the B-field intensities were about 100 times higher in the exposed enclosures. According to estimations of high-voltage line currents, the prevailing B-field intensities were regarded as fairly normal during summer conditions, although much higher intensities (possibly $20 \mu\text{T}$) could be expected under extreme circumstances. It may well be remembered, however, that the

computed total average 50-Hz E- and B-field intensities are rough estimates, based on several assumptions.

Plasma progesterone levels are commonly used to reflect ovarian activity. In the cyclic animal the progesterone level rises rapidly between approximately Days 3 and 12 of the cycle (Hansel et al., 1973). However, Henricks and Dickey (1970) and Hansel (1981) have suggested an increased progesterone secretion in the pregnant cow by the corpus luteum, due to an influence of the embryo from the 10th day of pregnancy and possibly earlier. Since an alleged effect of exposure to the high-voltage lines on reproductive performance possibly also would involve the progesterone secretion, and since it was not possible to detect pregnancy at such an early stage, the analyses of plasma progesterone levels were performed on blood samples collected mid-cycle, regardless of the heifers being inseminated or not.

The quality of the estrous detection routines is known to greatly influence the reproductive performance in a herd. Moreover, relations between the intensity of the signs of estrous and the conception rate and between weak estrous signs and a higher rate of returns have been shown to exist (Rottensten and Touchberry, 1957; Swensson and Andersson, 1980). Different symptoms occur in various frequencies and intensities at estrous in heifers (Esslemont et al., 1980). Therefore, in order to study the reproductive performance more closely, the weighted sum of systematically recorded symptoms at ovulation was used as a target parameter for intensity of estrous.

No effect on the prevalence of malformations was seen. However, it must be pointed out that the normal prevalence of malformations is very low. Therefore, this study would not supply evidence of even a rather large increase of this prevalence.

As can be judged from the analyses, the exposure of the heifers to the 400-kV, 50-Hz transmission line did not influence the length or variation of the estrous cycle, the mid-cycle plasma progesterone level, the intensity of estrous, the number of inseminations per pregnancy, the overall conception rate or the fetal viability. This is in agreement with the results from earlier studies (Algers et al., 1981; Algers and Hennichs, 1985).

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