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Extremely low-frequency electromagnetic field (EMF) generates alterations in the synthesis and secretion of oestradiol-17β (E_2) in uterine tissues: An *in vitro* study

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ABSTRACT

An electromagnetic field (EMF) of extremely low frequency may affect physiological processes in mammals. The aim of the present study was to determine the effect of an EMF on the synthesis and secretion of oestradiol-17β (E_2) in the porcine uterus. Endometrial and myometrial slices were harvested on days 12–13 of the oestrous cycle and exposed *in vitro* to an EMF (50 and 120 Hz, 8 mT) for 2 and 4 h in the presence or absence of progesterone (P_4). Subsequently, the incubation media were used to determine the concentration of E_2 with RIA. Tissues fragments were used to study the expression of *CYP19A3* mRNA using Real-Time PCR and the abundance of P450 aromatase using Western Blotting. The 50-Hz EMF increased E_2 release from the endometrium and the myometrium at both time points of *in vitro* incubation. A 120-Hz EMF decreased the endometrial secretion of E_2 after 2 h of incubation and did not affect E_2 secretion after 4 h. In the myometrium, the 120-Hz EMF increased E_2 secretion after 4 h of incubation. In P_4-treated uterine fragments, no significant EMF exposition-related changes were observed. Only myometrial fragments incubated in the presence of P_4 at 120-Hz EMF (4 h) released higher amounts of E_2 due to EMF treatment. The 50-Hz EMF exposure did not change the *CYP19A3* mRNA expression in endometrial fragments incubated in the presence or absence of P_4. In myometrial fragments, the highest *CYP19A3* mRNA expression was observed in fragments not exposed to the 50-Hz EMF and P_4-treated tissues compared to that in fragments exposed to 50 Hz EMF and incubated with or without P_4 and control (no EMF and no P_4) fragments. The EMF at 120 Hz decreased basal endometrial *CYP19A3* mRNA expression and did not change the expression in the P_4-treated endometrium. In the myometrium, the EMF at 120 Hz increased *CYP19A3* mRNA expression in slices incubated without P_4 and had no effect in the presence of P_4. The EMF exposure (50 and 120 Hz) did not affect P450 aromatase abundance in either the endometrium or the myometrium. In conclusion, the EMF induces changes in the synthesis and release of E_2 in uterine tissues harvested during days 12–13 of the oestrous cycle. These changes are related to the EMF frequency used, the time of the exposition and the presence of P_4. We suspect that this observed phenomenon might lead to changes in the intrauterine milieu of oestrogen, which is crucial for the proper activity of uterine tissues during the mid-luteal phase of the oestrous cycle.

1. Introduction

An extremely low-frequency electromagnetic field (EMF) is a state of expanse where each electric load or magnetic dipole is acted on by a specified force. In recent decades, the number of artificial sources of EMFs has rapidly increased, resulting in changes in the electromagnetic environment of the Earth. The EMF frequencies present in the environment ranges from extremely low (0–300 Hz) to GHz frequencies. Emitters of such fields include high-voltage lines, transformer stations, devices powered by industrial networks, car electric engines, magnetic resonance

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imaging, radar stations and mobile base stations. In addition, the use of EMF has steadily emerged in physical medicine therapies to treat, for example, inflammation of the abdominal organs [1].

The consequences of living organisms exposed to extremely low, non-ionising EMF generated in everyday life have been investigated since the 1960s. In addition to demonstrating the negative or positive impact of EMFs, previous studies also highlight the lack of influence of low-frequency fields on organisms. Cecconi et al. [2] examined the effect of EMF of extremely low frequencies on mouse pre-antral follicle development *in vitro* and demonstrated a significant decrease in follicular growth as a result of 33 Hz exposure but not for other studied frequencies, including 50 Hz. Chung et al. [3] showed that exposure to an EMF of extremely low frequency did not induce any unfavourable effects on spermatogenesis and reproductive capacity in human and animal models. Elbetieha et al. [4] showed no effect related to long-term exposure on the fertility of male or female mice.

In contrast, the first epidemiological studies provided worldwide have shown a correlation between electromagnetic radiation and the occurrence of diseases in humans [5]. In population of children living near high-voltage power lines, an increased risk of leukaemia was found [5]. Studies performed on small laboratory animal models indicated that the embryos of females exposed to EMFs are more susceptible to developmental disorders [6]. Previous studies have also shown that EMFs can lead to DNA synthesis disturbances and alterations in gene expression [7]. Moreover, studies on mammals documented the impact of EMFs on the length of the oestrous cycle [8], the fertilisation rate, the rate of embryonic development [6,9,10], and the endocrine disruptions and morphological parameters of reproductive organs [11–14]. The negative effects of EMF treatment on fertility were also observed in males [9,10,15–21]. The results showed that the consequences of EMF exposure might depend on its frequency and the time of exposure [2–24]. The effect of EMFs has primarily been studied in small laboratory animal models [2–4,9,11–15,17–21].

It was established that the porcine uterus possesses steroidogenic activity that is important to provide accurate conditions for the regulation of the oestrous cycle in non-pregnant pigs [25–27]. In cyclic pigs, oestrogen serves as the signal for the redirection of luteolytic prostaglandin F2α (PGF2α) from uterine vasculature to the uterine lumen [28]. Luteolysis in pigs is uterine-dependent and occurs during the last part of the luteal phase following stimulation of the uterine endometrium by progesterone (P_4) [29]. For days 10–12 of the oestrous cycle following stimulation of the uterine endometrium by P_4, luteolytic mechanisms start to develop, but until days 12–13 of the oestrous cycle porcine, CLs are refractory to luteolytic signals and do not possess luteolytic capacity [30–32]. Therefore, days 12–13 of the oestrous cycle are crucial for the CL to undergo luteolysis in response to PGF2α.

Since uterine E_2 has been identified as a crucial factor involved in the regulation of uterine activity, we aimed to determine the impact of EMF exposure on the synthesis and secretion of E_2 in the endometrium and the myometrium of pigs during days 12–13 of the oestrous cycle. To determine whether P_4 may affect the sensitivity of uterine tissues to EMFs, uterine slices were incubated *in vitro* in the presence or absence of P_4. We hypothesised that the EMF may induce alterations in the synthesis and secretion of E_2 in endometrial and myometrial tissues during the mid-luteal phase of the oestrous cycle, *i.e.,* days 12–13. These changes may be a result of disturbances in the mRNA expression of *CYP19A3*, which encodes a P450 aromatase that catalyses the formation of aromatic C18 oestrogen from C19 androgens [33,34]. Therefore, the aim of the present study was to determine how EMF (50 and 120 Hz, 8 mT) affects alterations in the synthesis and secretion of E_2 in uterine tissues harvested from pigs during the mid-luteal phase of the oestrous cycle, i.e., days 12–13, at the time when CLs are vulnerable to luteolytic factors.

2. Materials and methods

2.1. Animals and collection of uterine tissues

All experiments were performed on post-pubertal crossbred gilts (Polish Large White × Polish Landrace) weighing 90–110 kg that exhibited at least one oestrous cycle. Respecting the animal rights and rules of The Three Rs (3Rs), the number of animals per experimental group was minimised ($n = 4$). The gilts were housed on a private farm near Olsztyn under normal conditions (fed with normal diet according to Polish Norms 1993; water provided *ad libitum*) and subsequently sacrificed at a local commercial abattoir (WARMIA Biskupiec) within the standard operating procedure. Days 12–13 of the oestrous cycle were distinguished by the CLs morphology according to Akins and Morrisette [35]. Uteri were collected and placed in ice-cold PBS supplemented with streptomycin and transported to the Laboratory of Animal Physiology, University of Warmia and Mazury in Olsztyn, within 30 min. In the laboratory, each uterine horn was opened longitudinally on the mesometrial surface. The perimetrium was discarded by careful scraping using a scalpel blade, and fragments of the endometrium and the myometrium were collected with scissors and placed in PBS supplemented with 3% commercial antibiotic-antimycotic solution (Sigma Aldrich, St. Louis, MO, USA, LOT #105M4823V).

2.2. In vitro incubation of endometrial and myometrial slices

Individual endometrial and myometrial fragments were cut to 2-mm-thick slices weighing 95–105 mg and placed in separate wells of a 24-well culture plate. Subsequently, 1 mL of M199 medium (Sigma Aldrich, LOT #070M8310) supplemented with 0.1% bovine serum albumin (BSA) fraction V (Carl Roth GmbH + Co KG, Mühlburg, Karlsruhe, Germany, No. 8076.3) and 1% commercial antibiotic-antimycotic solution (Sigma Aldrich, LOT #105M4823V) was used. First, the slices were pre-incubated in a shaking water bath at 37 °C in an atmosphere of 95% O_2 and 5% CO_2 for 2 h. Then, the slices were incubated *in vitro* in fresh control medium or fresh medium supplemented with P_4 (10^{-5}M, Lot 13260, SERVA, Germany) in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C for 2 and 4 h. The medium components, incubation parameters and P_4 dose were selected according to references [26–28].

2.3. Electromagnetic field exposure system

The culture plates were placed in an EMF generated by the Astar generator (Magneris, Astar) equipped with flat applicators. The device generates an EMF with frequencies ranging from 2 to 120 Hz, with sinusoidal, triangular or rectangular waveforms. The properties of the EMF generated by the Astar generator, including electric and magnetic field properties and the relationship between them, were previously described by Koziorowska et al. [36]. The generator provides an opportunity to select the time of interaction and the value of magnetic induction. The tissue samples were exposed to a sinusoidal EMF at 50 Hz and 120 Hz (magnetic induction of 8 mT), separately. We selected these parameters since: 1) the EMF frequency of 50 Hz is ubiquitous in Europe [1], 2) EMF frequency of 120 Hz was found to affect cell cycle progression, and cell viability *in vitro* [37,38], 3) the typical exposure levels from magnetic field sources for industry and therapeutic equipment used in human medicine range from 1 to 16 mT [39]. Therefore, EMF at 50 Hz is the most prominent frequency in the environment and this is the most suitable frequency to study the effect of EMF on living organisms