Author’s response to reviews

Title: Anti-proliferative effect of extremely low frequency electromagnetic field on preneoplastic lesions formation in the rat liver

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Author’s response to reviews: see over
Dear editors of BMC Cancer journal,

Gladly and grateful we considered once more the suggestions of reviewers, and all new or modified paragraphs are showed highlighted in blue throughout the manuscript.

Reviewer Comments:

Referee 1
Reviewer: Santi Tofani

Major Compulsory Revisions:
The paper is now more complete. Even though the new additional info show that the experimental design for the sham exposure group was not well done. The + 3°C of temperature increase experienced by the exposure group in respect to the sham one may be considered as a confounding factor that may or may not influence the observed results. This aspect should be reported in the discussed with the corresponding comments by the Authors.

For this suggestion, the differential of temperature could have or not an affect on the results; however the corporal rat temperature is at 37.5°C, which is biologically regulated by the animal, while the highest registered temperature was 27°C in our experiments. We can not discard that in our experiment the temperature differential between comparatives groups, has not effect. The difference of temperature was due to turn off the solenoid when the sham-group was exposed. For new experiments we already are using bifilar coils.

To supplement information on the temperature difference between the groups the in the subtitle “Experimental design” of the “Methods” section and in the subtitle “General observations” of the “Results” section we included the following sentence:

Experimental design
…The first group, which was used as the normal control did not receive treatment, NC group. The second group, which was used as the positive control for carcinogenesis, was subjected to the carcinogenic treatment and was kept under the same stress conditions without electromagnetic field exposure (sham-exposure) throughout the experiment; in this case the equipment was turned off, CT group. The third group, additionally to the carcinogenic treatment, was exposed to ELF-EMF according to “Animal exposure” section, CTF group.

General observations
The average body and relative liver weights of rats treated either with CT or CTF were not different throughout the experiment (data not shown). We also monitored the temperature of sham-exposure (CT) or exposure (CTF) groups during the 32 days and their values were recorded at specific times shown in Table 1. The three degree differential temperature between the groups was due to the solenoid was turned off when the sham-group was exposed.
A less important issue is that related to possible mechanism of action. Considering the intensity of magnetic fields (above 1mT) a possible influence on radical chemistry should be not rule out. Also this matter should be reported in the discussion.

We agree with this suggestion, the intensity of magnetic fields could interfere on radical chemistry. In the “Discussion” section we already included a possible action mechanism of electromagnetic field at calcium modulation level, considering that it could disrupt the electrochemical balances through membranes; nevertheless we cannot speculate more about other mechanism at more fine level. Furthermore, currently we are designing new experiments to investigate this important topic, including studies at level of radical pairs mechanism of electron transfer rates.

The possible mechanism that we included, you can see below:

Furthermore, evaluations in experimental models have been established that the electromagnetic fields are able to modulate the intracellular calcium (Ca2+) when cellular homeostasis is disrupted [34]. Calcium is a highly versatile intracellular signal that can regulate many different cellular functions, whether normal or pathological; thus, the consequences of Ca2+ signaling depend on steady state between Ca2+ influx, efflux, and storage [35]. Cancer development takes place through rapid proliferation and the continuous increase of altered cells that modify the cellular environment [19], including the flow of ionic charges across the cell membrane, such as Ca2+ flow. Given that several blockers of Ca2+ entry inhibit tumor growth [36], we cannot discount that ELF-EMF could be regulating Ca2+ flow in the cells.

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

For this comment; initially, we did the statistics evaluation including student-\(t\) and ANOVA tests, and our significances were very similar in both of them. Finally we decided to show student-\(t\) test because ANOVA test is used for multi-comparisons.

Referee 2
**Reviewer:** Ronald J Midura

The authors are commended for their diligence and efforts in adequately revising their manuscript based on the original critiques.

Dear Dr. Midura, thank you very much for your interest no doubt you suggestions and comments improved substantially our manuscript.

Referee 3
**Reviewer:** Luciana Dini

The revised form of the manuscript entitled “Anti-proliferative effect of extremely low frequency electromagnetic field on preneoplastic lesions formation in the rat liver” by Jiménez-García and co-Authors has been corrected and /or explanations were given according to the suggestions of the referees.
Before the manuscript is published I suggest to include the name of the firms in the materials and methods section.

In the subtitle of “System of ELF-EMF exposure” and “TUNEL assay and immunohistochemical analysis” of the “Methods” section we included the names of the firms that we ignored.

System of ELF-EMF exposure
A uniform and homogeneous ELF-EMF of 120 Hz was generated by a solenoid coil with 1900 turns in two layers and with AWG 20-gauge copper-enameled magnetic wire in a diameter of 25 cm, which was driven by an alternating current source (Staco Variac variable transformer 511, ISE Inc; Cleveland, OH, USA). The equipment was controlled by a computer. The coil generated 0.1 – 4.5 mT in its center, where the rats received the exposure. The magnetic flux density was measured using a Gauss/Teslameter (Hall effect gaussmeter F.W. BELL 5070, SYPRIS Test & Mesurements; Orlando, FL, USA); the signal parameters were monitored by an inductive coil that was connected in parallel to a resistance by an oscilloscope (Tektronix TDS2024, TEKTRONIX Inc; Beaverton, OR, USA). The current flow was measured by an alternating current meter that was connected in series with the solenoid (Digital multimeter Tech TM-178, Techman Electronics Inc; La Verne, CA, USA). When the animals were exposed or had sham-exposure, the temperature inside the solenoid was monitored by a temperature sensor (LM35 IC: Integrated Circuit, National Semiconductor Corporation Americas; Santa Clara, CA, USA). The temperature sensor location and the shape of signal wave are shown in Figure 2.

TUNEL assay and immunohistochemical analysis
...Tissues images were captured by optical microscopy (Olympus 1X70, Olympus Europa GmbH, Hamburg, Germany).