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Multifractal dimension and lacunarity of yolk sac vasculature after exposure to magnetic field

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ABSTRACT

Several studies have reported about the effects of magnetic fields (MFs) on vascular tissue. Extremely low frequency magnetic fields (ELF-MFs) can promote either inhibition or stimulation of vasculogenesis and angiogenesis, depending upon the intensity and time of exposure to the MF. To investigate the possible effects of ELF-MF on vascular processes, it is necessary to employ methods that allow parameterization of the vascular network. Vascular network is a structure with fractal geometry; therefore, fractal methods have been used to evaluate its morphometric complexity. Here, we used the lacunarity parameter (complementary method of fractal analysis) and multifractal analyses to investigate angiogenesis and vasculogenesis in the embryonic yolk sac membrane (YSM) of Japanese quails (*Coturnix japonica*) with and without exposure to an external MF of 1 mT and 60 Hz. Lacunarity results showed that the vascular density was lower for the group exposed to the magnetic field for 9 h/day. In addition, multifractal analysis showed reduced vascularization in the experimental groups (6 h/day and 9 h/day of exposure to MF). Furthermore, multifractal analysis showed difference between the groups exposed for 12 and 24 h/day. Using multifractal methods (generalized dimensions and singularity spectrum), it was possible to characterize the vascular network of the quail embryo YSM as a multifractal object, therefore proving this method to be a more appropriate application than the traditional monofractal methods.
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Introduction

The effects of electric field (EF), magnetic field (MF) and electromagnetic field (EMF) on organisms, focusing on various cells and tissues, are of great interest to researchers. Several studies report the effects of MF on vascular tissue (McKay et al., 2007). Static magnetic fields (SMFs), depending on their intensity, can either promote the proliferation of human umbilical vessel endothelial cells (HUVECs) (Martino et al., 2010), or can prevent the growth of those very cells (Li et al., 2007). Extremely low frequency electromagnetic fields (ELF-EMF) are capable of stimulating proliferation, migration and endothelial tube formation (Delle Monache et al., 2008). Furthermore, studies have also shown that ELF-EMF produces vasodilatation, vasoconstriction and alterations in angiogenesis and vasculogenesis (Tepper et al., 2004; McKay et al., 2007; Bekhite et al., 2010). The MF and the EMF can inhibit (Costa et al., 2013) or stimulate vasculogenesis (Tepper et al., 2004; Bekhite et al., 2010) and can inhibit (Ruggiero et al., 2004; Wang et al., 2009; Balanezhad et al., 2010) or stimulate angiogenesis (Roland et al., 2000).

Both vasculogenesis and angiogenesis are involved in growth, wound healing, tumors and several diseases (Folkman, 1989; Carmeliet, 2003; Folkman, 2007; Boscolo and Bischoff, 2009; Liu et al., 2012). The effects caused by MF or EMF on the vessels indicate that these effects can be used as alternative therapies against cancer and other diseases related to vasculogenesis and angiogenesis (Bassett, 1993; Cameron et al., 2007; Ishida et al., 2008).

The vascular network can be considered to be fractal due to its geometric complexity and ramified vascular structure characterized by self-similarity, which means that a portion of that structure is statistically similar to the whole (Mandelbrot, 1983; Bassingthwaighte et al., 1994). This implies that the properties of the structure do not change with the scale of observation, thus it is characterized by scaling (Telesca, 2007). Mathematically, scaling implies that the statistics used to describe the fractal object behaves as a power-law function of the scale, with an exponent called fractal dimension as the key parameter that quantitatively describes a fractal (Mandelbrot, 1983; Bassingthwaighte et al., 1994).

Several studies have used fractal dimension to measure the behavior of vascular network in diseases such as retinopathies (Avakian et al., 2002; Cheung et al., 2009; Kunicki et al., 2009), and for the study of the effects of drugs on vascular growth (Parsons-Wingerter et al., 2006; Mckay et al., 2008; Výboh et al., 2010) and tumor angiogenesis (Kirchner et al., 1996; Taverna et al., 2009). Nevertheless, fractal dimension estimation has two hindrances: firstly, it refers to the amount of space filled by the object under study without describing the organization of such object in the space, hence different fractal objects may have

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Table 1
Experimental groups, exposure time to the magnetic field (MF) and total exposure to MF per day.

	Control group	Group 1	Group 2	Group 3	Group 4
Number of eggs	20	20	20	20	20
Exposure to MF (h)	–	2	3	4	24
Total exposure/day (h)	–	6	9	12	24

the same fractal dimension value (Mandelbrot, 1983; Gould et al., 2011); secondly, the method to obtain fractal dimension is related to one single dimension, and as some fractal objects present different fractal properties in their different regions, it is therefore necessary to represent them by using more than one dimension (Stanley and Meakin, 1988).

To solve the first question, lacunarity, which is a way to calculate how the fractal object is organized in space, can be used (Mandelbrot, 1983; Gould et al., 2011). This parameter measures the distribution of holes or gaps throughout the object embedded in the image, and provides complementary analysis to the methods of fractal dimension. Regarding the second question, an object with characteristics mentioned above is known as a multifractal object, represented by various fractal dimensions through the generalized dimension spectrum, as well as the singularity spectrum.

Costa et al. (2013) used the fractal dimension method (box-counting and information dimension), and showed the inhibition of vasculogenesis and angiogenesis in the YSM of quail embryo (*Coturnix japonica*) when exposed to some extremely low frequency magnetic field doses (ELF-MF). Our aim is to test whether lacunarity and multifractal analysis are able to identify any changes in the YSM vasculature of quail embryo, which were not detected by the monofractal analysis.

Materials and methods

All animals in the experiments were used according to the protocol (number 028/2012-012531/2011-E09/CEUA-UFRPE) approved by the Ethics Committee on Animal Use (CEUA) of the Rural Federal University of Pernambuco. The experimental protocol was performed as described in Costa et al. (2013). Briefly, YSMs of quail embryos were obtained, exposed to MF, and YSM imaging and image processing were performed. After 2 days of incubation, a window of approximately 3 cm^2 was created in the egg shells, and 2.5 ml of albumen was removed. Then, the opening was covered with PVC film (polyvinyl chloride). This window enabled the visualization of YSM and its direct exposure to the MF. Within 48–96 h of incubation, macroscopic expansion of the YSM vascular network was clearly observed.

Exposure to MF was performed with a pair of Helmholtz coils (PHYWE, Göttingen, Germany), and the MF intensity was measured by a teslameter (PHYWE, model 13610.93) connected to a probe (PHYWE, model 13610.02) placed on the horizontal axis of each Helmholtz coil. The eggs were placed along the horizontal axis between the coils for uniform exposure to MF, with an intensity of 1 mT.

Five groups of 20 eggs each were used, each group being exposed to MF at different times, under the same experimental conditions, with the same incubator and pair of Helmholtz coils. The eggs were placed in the incubator for 2 h after being laid, and remained in the incubator between two Helmholtz coils for 96 h. For the control group, the coils were switched off. All groups (except the control group) were exposed to MF from 48 h of incubation. Group 1 was exposed to MF for 2 h, three times a day, with intervals of 6 h between applications, resulting in a total exposure of 6 h/day. Group 2 was exposed to MF for 3 h, with an interval of 5 h between applications, amounting to 9 h/day. Group 3 was exposed for 4 h, with an interval of 4 h between applications (total of 12 h/day). Group 4 was exposed for 24 h continuously. Table 1 shows the organization of groups in relation to MF exposure.

After 72 h of incubation, the YSM vascular network was photographed with a digital camera (Sony DSC W-130, San Diego, CA, USA). The visual field was limited by the size of the egg, especially by the egg minor axis, which ranged from 2.39 to 2.62 cm (Fig. 1A). The images of the YSM vascular network (1920 × 1080 pixels) were manually skeletonized (Fig. 1B) using Microsoft Paint program to be evaluated by the lacunarity parameter and the multifractality analysis. The same procedure was repeated at 96 h of incubation.

Lacunarity analysis

We used Image J software (Wayne Rasband, National Institutes of Health in Bethesda, Maryland, USA) with FracLac plug-in (A. Karperien – Charles Sturt University, Australia) to obtain the lacunarity values (distribution of gaps in an image). Lacunarity is related to the pixel distribution of an object in an image. The pixel count of the image is obtained, covering the skeletonized vasculature with a series of grids, each grid containing a number of boxes with different sizes (ε) and with different orientations (g).

The mean value of the lacunarity is calculated as follows:

$$\Lambda = \left[\sum_{g}\sum_{i}\left(1+(\sigma|\mu)^2\right)\right]/n \qquad (1)$$

where σ is the standard deviation and μ is the mean pixel value per box at a side size ε, and n is number of box sizes in a box count at an orientation g. The sum is made over all values of ε and g.

Multifractal analysis

Image J with plug-in FracLac was used to carry out the multifractality analysis. This analysis is based on the generalized dimensions spectrum, represented by D_q, which is dependent on variable q. Variable q is the exponent, which expresses the fractal properties in different scales to an object (Stošić and Stošić, 2006), $q \in (-\infty, +\infty)$. For a multifractal

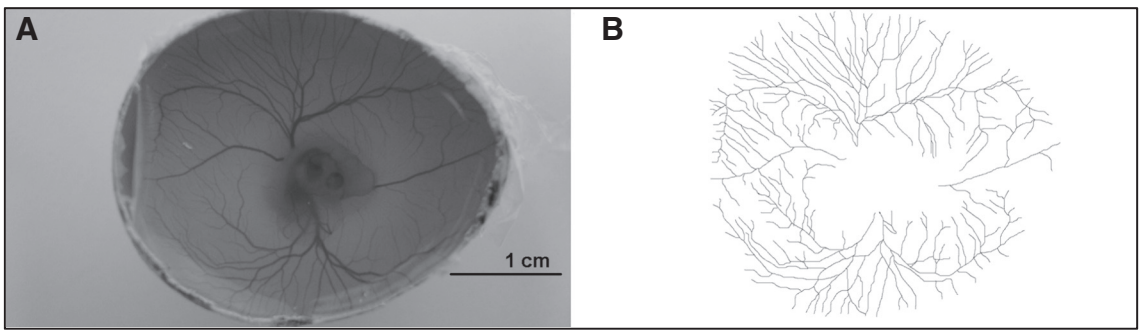

Fig. 1. Extra-embryonic YSM vascular network and the corresponding skeletonized image.

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