

## Research Article

# Mouse Embryo Development under an Enriched Atmosphere of negative Air Ions: A Pilot Study

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## Keywords

Controlled negative air-ion generator (CNAIG); Mouse embryo development; In vitro fertilization; Embryo culture

## Abstract

Several studies have shown that negative air ions may have positive effects on the living organisms. The aim of our study was to evaluate whether an enriched environment of negative air ions have an influence on the mouse embryo development. A controlled negative air-ion generator (CNAIG) was installed in a standard incubator and test embryos cultured under the enriched atmosphere with negative ions. Embryo development was judged in terms of blastocyst rates, blastocyst total cell number, and potential of these to develop to term.

In a first series of experiments, we observed that mouse embryos cultured in the atmosphere enriched with negative ions developed up to the blastocyst stage with a 96.1% (49/51) efficiency, similarly ( $p = 0.675$ ) to controls 97.1% (32/34). Blastocysts obtained in the experimental group showed a total mean number of cells ( $110 \pm 20.1$ ) statistically equivalent ( $p = 0.351$ ) to controls ( $121 \pm 33.4$ ) cultured under standard conditions. In a second series of experiments, blastocysts were transferred to synchronized pseudo-pregnant females, so embryo development could be followed *in vivo*. A total of 33.3% of the blastocysts transferred from the experimental group developed to term, in a similar proportion ( $p > 0.05$ ) to the control group (40%). In this pilot study, we demonstrate for the first time that mouse embryos cultured in an enriched negative ions atmosphere are able to develop *in vitro* and *in vivo* with very high successful rates.

## ABBREVIATIONS

CNAIG: Controlled Negative Air Ion Generator; ICM: Inner Cell Mass; TE cells: Trophectoderm cells

## INTRODUCTION

Negative air ions are negatively charged particles in the air that are naturally formed by cosmic rays, lightning, waterfalls, rains or wind action [1,2]. The effects of the negative air ions on the living organisms have been studied since the beginning of the 20<sup>th</sup> century. The initial experiments of Kiyanitsyn I.I. [1,2], confirmed by Chizhevsky in 1995 [3], and lately reproduced by Goldstain and Arshavskaya in 1997 [4], have showed the vital role of negative air ions on the living organisms. These experiments showed that the animals died under the atmosphere conditions with absence of negative ions. Also, deficiency of negative air ions has been demonstrated to cause fatigue, migraine, depression, behavior changes and respiratory symptoms in humans [5]. In opposite, many beneficial effects of negative air ions, such as: removal of the bacteria, increase of the oxygenation of blood, improve alertness, metabolism, immunity and proved healing effects on allergy, asthma, insomnia and depression have been described [6-8].

Since air purifiers produce high amount of negative air ions

by the high-voltage electrical discharge, they have been used for air purification in many offices, public facilities and hospitals in Europe and Russia [9]. It has been shown that negative air ions purify the air from bacteria, mold and allergens. Since 1978, from the first successful IVF trial, the rapid development of assisted reproduction technologies has shown that the success directly depends on the air quality of the IVF laboratories or incubators [10]. So far, many different systems have been designed for purification of the chemicals, gasses, odors, particles and biological compounds in the laboratories air [11]. The technology of air purifiers which are based on electric discharge can release high concentrations of negative ions. These negative ions are able to attach themselves to airborne toxins and remove all the pollutants from the air [12]. The presence of abundant amounts of negative air ions in the nature are an indication for clean air, such as *'Niagara and Yosemite Falls'*, the natural producers of 100.000 negative ions/cm<sup>3</sup>, that are considered for the healthiest environments in the world [13].

Although, as previously mentioned, many scientific studies have been demonstrating the beneficial effects of high levels of negative ions, to our knowledge the influence of negative ions on embryo culture remains unexplored. The aim of this pilot study was to evaluate, for the first time, the influence of an enriched environment of negative ions on mouse embryo development.

## MATERIALS AND METHODS

### Animals

Animal care and procedures were conducted according to protocols peer approved by the Ethics Committee on Animal Research (DAMM-7436) of the Parc Científic de Barcelona (PCB), Spain. Hybrid (B6/CBA) and out bred CD1 females, 5-6 weeks of age (25-30 g), and male mice from the same genetic strain, 8-10 weeks of age (25-30 g), were purchased from Janvier Laboratories. All animals were used within 4-6 weeks after reception. Upon arrival, all mice were quarantined and acclimated to the PCB Animals facility (PRAL) for approximately 1 week prior to use. Mice were housed three to four per cage in a room with a 12 h light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water.

### Embryo collection and culture

Fresh one-cell mouse embryos were collected from super ovulated females (B6CBAF1 strain), previously mated with males from the same genetic background. After collection, good morphology embryos showing two pronuclei and first and second polar bodies were selected, washed thoroughly, and placed in culture dishes. Embryo culture was performed in 35mm *nunc* dishes with approximately 30  $\mu$ L micro droplets of culture medium (Global supplemented with 10% (v/v) LGPS, LifeGlobal) and covered with mineral oil (LifeGuard, LifeGlobal) and prepared the day before use. Control embryos were cultured in a standard three gas incubator under optimal conditions: 37.3°C, 7.0% CO<sub>2</sub> and 7.0% O<sub>2</sub>. In the experimental group, culture dishes were placed under the same conditions as the control group, with the exception on that the incubator atmosphere was enriched with Negative Air-Ions controlled by a CNAIG (Controlled Negative

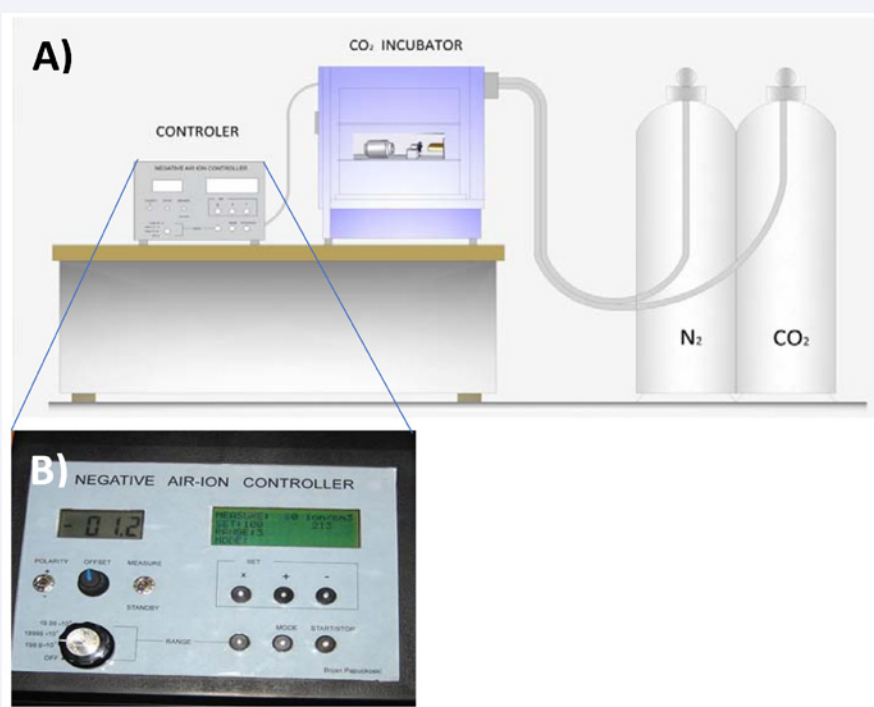
Air-ion Generator) installed on the central shelf (Figure 1). The CNAIG was set to generate a concentration of 100.000 of Air-ions /cm<sup>3</sup> of air. Embryo development was followed every 24 hours up to Day 5/6. Blastocysts were selected for either transfer into pseudo pregnant recipients or for fixation for total cell counts.

### Embryo transfer

Two point five (2.5) days after the vaginal plug detection, CD1 females crossed with vasectomized males were randomly assigned to the control or experimental group. Embryo transfers were performed non-surgically using a commercial non-surgical embryo transfer (NSET, Paratechs) protocol. Briefly, an NSET device was coupled to a P2 pipette with volume adjusted to 1.8  $\mu$ L. Blastocysts were loaded in each device within a culture medium droplet under a stereomicroscope. After loading the blastocysts, the volume in the P2 pipette was re-adjusted to 2  $\mu$ L to create an air bubble and to avoid the loss of the embryos by capillarity. The recipient female assigned for transfer was then immobilized, and a NSET small speculum was carefully introduced in the vagina. With the animal still immobilized, the NSET device loaded with the embryos was introduced by the speculum through the cervix. When the base of the device got in contact with the speculum, the blastocysts were transferred by pressing the plunger of the P2 pipette up to the end. Having the plunger of the pipette still pressed, NSET device was removed and checked under the stereomicroscope to confirm that all embryos had been correctly transferred. Finally, the speculum was removed and the female returned to its corresponding cage.

### Blastocysts fixation and processing for immunofluorescence analysis and cell counts

Blastocysts from control (n= 29) and experimental (n= 20)



**Figure 1** Schematic representation (A) of the controlled negative air-ion generator (CNAIG) and its real appearance (B).

groups were fixed and extracted for 30 min at 3°C in a microtubule stabilizing buffer (MTSB-XF). Once fixed, blastocysts were stored until processing at 4°C in a phosphate-buffered saline (PBS) blocking solution [14]. Once fixed, the blastocysts were washed in PBS blocking solution, incubated in Hoechst 33258, and finally put on a mounting solution droplet on a glass slide, as described elsewhere [14].

### Microscope analysis

Once stained and mounted, the blastocysts were examined using an epifluorescence microscope (Nikon E1000) fitted with specific filters for Hoechst, Fluorescein and Texas Red and a 50W mercury lamp. Digital images were acquired with a Nikon software and analyzed with Image J software for total cell counts.

### Statistical analysis

Data were analyzed by *chi-square test* to compare *in vitro* development and full term developmental rates and *students' t* test to compare total mean number of cells between the control and experimental group. A probability value of  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

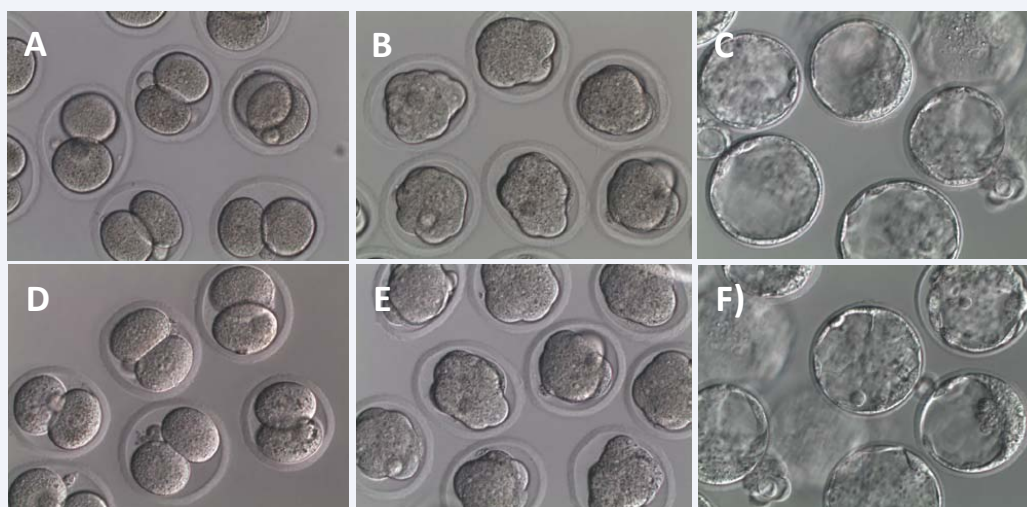
In the first series of experiments, we wanted to evaluate the influence of the experimental conditions on the ability of the mouse embryos to develop *in vitro*. Embryos cultured in the atmosphere enriched with negative ions developed up to the blastocyst stage with a 96.1% (49/51) efficiency, similarly ( $p = 0.675$ ) to controls 97.1% (32/34) (Figure 2). Afterwards, we performed total cell counts on the blastocysts obtained, as this is a strategy commonly used in basic research to better ascertain the quality of blastocysts produced. Blastocysts in the experimental group revealed a total mean number of cells ( $110 \pm 20.1$ ) on ICM and TE cells, statistically equivalent ( $p = 0.351$ ) to controls ( $121 \pm 33.4$ ) cultured under standard conditions (Figure 3). The results obtained in this first series of experiments showed that the presence of negative air ions in the culture atmosphere does not seem to have a detrimental effect

the early embryo development, as reflected in the blastocyst rates and quality of the blastocysts judged by the total cell counts. These results prompted us to further explore the potential of the blastocysts obtained to develop *in vivo*. A total of 4 out of 12 (33.3%) blastocysts transferred from the experimental group into synchronized pseudo-pregnant females were able developed to term. This proportion was similar ( $p = 0.706$ ) to the percentage of pups obtained in the control group (40%, 8/20). All pups obtained both in the experimental and control groups apparent to be healthy and presented a birth weight statistically similar ( $p = 0.254$ ) among the two groups ( $1.975 \pm 203.0$  and  $1.856 \pm 199.4$ , respectively).

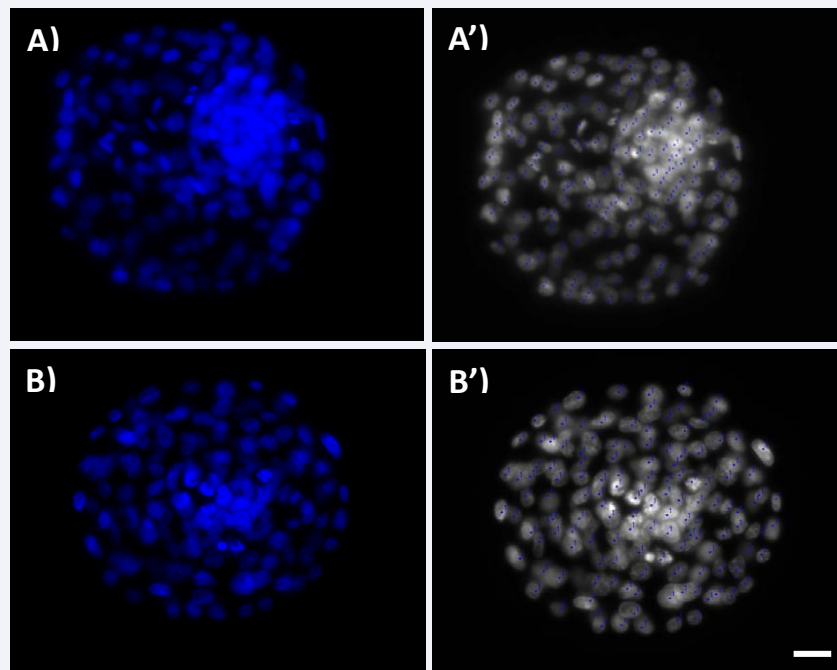
Previous studies have demonstrated the beneficial effects of negative air ions when applied *in vivo* for the treatment of animals, herbs or humans. To our knowledge, the beneficial effects of negative ions on embryo culture procedures where a purified environment is used had not been previously explored. In these experiments, we demonstrate, for the first time, that, under a controlled concentration of negative ions, mouse embryos are able to develop *in vitro* and *in vivo* up to term with high success rates.

## CONCLUSION

Our experiments show that mouse embryos cultured *in vitro* under the controlled presence of negative air ions are able to develop efficiently up to the high-quality blastocysts. Furthermore, *in vivo* studies carried out revealed that the embryos cultured during the pre-implantation period under the enriched environment with negative air ions once transferred into pseudo-pregnant females can develop up to term and result in apparently healthy offspring, as no abnormalities were registered in the mice produced. While this is a pilot study and thus conclusions must be drawn carefully, the results obtained are promising, as the generation of a controlled negative air ion atmosphere could represent a good strategy to eventually improve the air quality of the laboratories or incubators currently used for the culture of human embryos.



**Figure 2** Mouse embryo development shown at two-cell, morula and blastocyst stages. In the upper part of the figure (A-C), the mouse embryos cultured under standard conditions (control) and in the bottom row (D-E) the embryos cultured under the enriched atmosphere of negative air ions.



**Figure 3** Mouse control (A-A') and tested (B-B') blastocysts fixed and stained for total cell counts with the visible nuclei shown in blue (A and B) and the corresponding raw images (A' and B') used for cell counting with ImageJ software.

## REFERENCES

1. Bailey WH, Johnson GB, Bishop J, Hetrick T, Su S. Measurements of charged aerosols near  $\pm$  kV DC transmission lines and in other environments. *Leee T Power Deliver.* 2012; 27: 371-379.
2. Annegarn HJ. Electrical properties of the indoor atmosphere of the Carlton Center. 1979. In *Proceedings International Conference on Air Pollution*, CSIR, Pretoria, South Africa.
3. Chizhevsky AL. On the shore of the Universe. Unexplainable phenomenon. *Mysl, Moscow.* 1995; 41-73.
4. Goldstain NI, Arshavskaya Z. Is atmospheric superoxide vitally necessary?. *1997; 52: 396-404.*
5. Sulman FC, Levy D, Pfeifer Y, Superstine E, Tal E. Airionometry of hot, dry desert winds (Sharav) and treatment with air ions of weather-sensitive subjects. *Int J Biometeorol.* 1974; 18: 313-318.
6. Krueger AP, Kotaka S, Reed EJ. The course of experimental influenza on mice maintained in high concentrations of small negative air ions. *Int J Biometeorol.* 1971; 15: 5-10.
7. Tom G, Poole M F, Galla J, Barrier J. The influence of negative air ions on human performance and mood. *Hum Fact.* 1981; 23: 633-636.
8. Ben-Dov I, Amirav I, Shochina M, Amitai I, Bar-Yishay E, Godfrey S. Effect of negative ionization of inspired air on the response of asthmatic children to exercise and inhaled histamine. *Thorax.* 1983; 38: 584-588.
9. Cohen J, Gilligan A, Esposito W, The Ministry of Health of the Russian Federation (MHRF): Sanitary and epidemiological norms on hygienic requirements to ionic air formula for industrial and public quarters. Moscow, Russia: Russian Ministry of Health Protection. 2003; 2: 1294-1303.
10. Schimmel T, Dale B. Ambient air and its potential effects on conception *in vitro.* *Hum Reprod.* 1997; 12: 1742-1749.
11. Boone WR, Johnson JE, Locke AJ, Crane MM, Price TM. Control of air quality in an assisted reproductive technology laboratory. *Fertil Steril.* 1999; 71: 150-154.
12. Krueger AP, Smith RF, Ing Gan Go. The action of air ions on bacteria. Protective and lethal effects on suspensions of staphylococci in droplets. *J Gen Physiol.* 1957; 41: 359-381.
13. Ryushi T, Kita I, Sakurai T, Yasumatsu M, Isokawa M, Aihara Y, et al. The effect of exposure to negative air ions on the recovery of physiological responses after moderate endurance exercise. *Int J Biometeorol.* 1998; 41: 132-136.
14. Costa-Borges N, Paramio MT, Calderón G, Santaló J, Ibáñez E. Antimitotic treatments for chemically assisted oocyte enucleation in nuclear transfer procedures. *Cloning Stem Cells.* 2009; 11: 153-166.

### Cite this article

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