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# Sex-related differences in lung inflammation after brain death

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ABSTRACT

*Background:* Donor sex has been suggested to be a factor influencing organ transplantation outcome. Sex hormones possess inflammatory and immune-mediating properties; therefore, immune responses may differ between males and females. Brain death (BD) affects organ function by numerous mechanisms including alterations in hemodynamics, hormonal changes, and increased systemic inflammation. In this study, we investigated sex-dependent differences in the evolution of lung inflammation in a rat model of BD.

*Materials and methods:* BD was induced by a sudden increase in intracranial pressure by rapidly inflating a balloon catheter inserted into the intracranial space. Groups of male, female, and ovariectomized (OVx) female rats were used. Lung vascular permeability, inducible nitric oxide synthase, and intercellular adhesion molecule 1 expression were analyzed 6 h after BD. Serum female sex hormones, vascular endothelial growth factor, and cytokine-induced neutrophil chemoattractant 1 levels were also quantified. Lung sections were analyzed by histology.

*Results:* After 6 h of BD, serum estradiol and progesterone concentrations in female rats were significantly reduced. Lung microvascular permeability was increased in females compared to males. Cytokine-induced neutrophil chemoattractant 1 and vascular endothelial growth factor concentrations were increased in female rats compared to males. Furthermore, female rats showed higher levels of leukocyte infiltration and inducible nitric oxide synthase expression in the lung parenchyma.

*Conclusions:* Our results indicate that the more severe lung inflammation in female animals after BD might be related to acute estradiol reduction. Based on our findings, we believe that, in a future study, a group of female treated with estradiol after BD could indicate a possible therapy for the control of lung inflammation in the female donor.



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## 1. Introduction

The impact of brain death (BD) on organs that might be used for transplantation is complex and includes alterations in hemodynamics and inflammatory and hormonal changes. Currently, many issues are considered in donor lung evaluation including donor and recipient comorbidities, ABO compatibilities, cytomegalovirus serology, and size matching. However, donor and recipient sex are neither directly considered nor matched. A few studies have assessed sex matching in lung transplantation, but they examined limited sample sizes [1,2]. Several groups have reported that donor sex influences the outcome of organ transplantation, and survival after transplantation of liver, kidney, and heart is decreased when a female organ is transplanted in a male recipient [3–5]. With regard to lung transplants, the literature is controversial. There is evidence that any association between donor–recipient gender mismatch and improved survival occurs [6] and also that transplantation with donor–recipient gender mismatch is associated with delayed histologic obliterative brochiolites and improved survival [7]. Other studies conclude that female donor to male recipient graft survival is lower compared to that of other sex combinations [1], and female sex was found to be one of the donor characteristics related with short-term and long-term mortality [8].

Female sex hormones are involved in maintaining the functional activity of organs and systems; therefore, they are potential modulators of inflammation after BD. Although there is no convincing explanation for the clinical disadvantage of female-to-male organ transplantation, the hormonal milieu has been proposed as a possible contributor [9]. The protective effects of estrogen in ischemia-reperfusion injury [10,11] and wound healing have been documented for various organs. Opposite effects of androgen have also been described in immune response and wound healing [12].

Because BD damages lung function by inducing hemodynamic, inflammatory, and neurohumoral mechanisms [13] and sex hormones possess inflammatory and immune-mediating properties, we investigated the sexual influence on lung inflammatory processes in a rat model of BD.

## 2. Materials and methods

### 2.1. Study groups

Rats were assigned to three groups ($n = 12$ each) as follows: (1) female rats in the proestrus or estrus phase of the estral cycle, (2) ovariectomized female (female-OVx) rats, and (3) male rats. Estrous cycle phases were determined by morphologic features of cells in vaginal smears and quantifying uterine weight. All animals were subjected to BD.

### 2.2. Animals

Female and male Wistar rats (200–250 g) from our institutional animal facilities were used. They were housed in groups of three rats per cage (12-h light-dark cycle, 21 ± 2°C) with free access to food and water. All animals received humane care, and the experiments were approved (report no. 0344/12) by the local Animal Care Committee.

### 2.3. Rat model of BD

The animals were anesthetized with 5% isoflurane, intubated and ventilated with a volume control ventilator (Harvard Apparatus, Inc., Holliston, MA) with a tidal volume of 10 mL/kg, frequency of 70 breaths/min, and oxygen fraction of 100%. All animals were monitored for 6 h through a catheter in the carotid artery, and the jugular vein was catheterized for fluid administration (saline solution, 2 mL/h).

A 1-mm hole was drilled through the skull, and a Fogarty-4F catheter (Baxter Healthcare Co., Irvine, CA) was inserted intracranially. To raise intracranial pressure and induce BD, the balloon was rapidly inflated with 400–500 μL saline solution. BD was confirmed by maximally dilated and fixed pupils, apnea, absence of reflexes, and a drop in mean arterial pressure (MAP). After BD, the anesthesia was interrupted.

### 2.4. Ovariectomy

Ten days before BD induction, female rats were anesthetized with ketamine and xylazine (100 and 20 mg/kg, respectively). An incision was made on the lower part of the abdomen; the ovaries were identified, held tightly, and removed. The incision was sutured, and each animal received a single intramuscular injection of Pentabiotic (570 mg/kg; Fort Dodge, Sao Paulo, Brazil). OVx effectiveness was confirmed by vaginal smears and measuring the uterine weight.

### 2.5. Quantification of serum levels of female sex hormones and corticosterone

Serum levels of estradiol and progesterone were determined using radioimmunoassay kits (Coat-A-Count; Siemens, Berlin, Germany). Corticosterone concentrations in serum were determined using an enzyme immunoassay kit according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI).

### 2.6. Blood gases, electrolyte and lactate levels, hematocrit, and white blood cell counts

Using a gas analyzer (Radiometer ABL 555; Radiometer Medical, Copenhagen, Denmark), blood gases and electrolyte and lactate levels were measured at baseline (0 min) and 3 h or 6 h after BD induction. Blood samples at the same time points were used for quantifying the hematocrit and white blood cell counts using an automatic hematology analyzer (Mindray BC 2800 Vet, Shenzhen, China).

### 2.7. Lung myeloperoxidase activity

Myeloperoxidase (MPO) level was measured as an index of the presence of neutrophils in lung tissue. Samples were prepared as described elsewhere [14]. Tissue homogenates with phosphate buffered saline (PBS) containing 0.5% hexadecyltrimethylammonium bromide and 5-mM ethylenediamine tetraacetic acid (pH 6.0, 3 mL/kg) were incubated for 15 min with $H_2O_2$ and o-Dianisidine (Sigma, St. Louis, MO), and the