ORIGINAL ARTICLE

The influence of female sex hormones on lung inflammation after brain death - an experimental study

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SUMMARY

Organ donor's age negatively influences graft survival of organs, increasing risk of complications. Aging occurs in both men and women; however, the menopause marks a decrease in sex hormones and a sudden increase in the process of vascular aging. We investigated sex hormones' influence on the lung inflammatory process induced by BD in female rats. Wistar rats were grouped as: female rats from high estradiol to heat period (non-OVx) and ovariectomized (OVx) female rats. Ovariectomy was carried out 10 days before BD. BD was induced using intracranial balloon rapid inflation. Serum hormones and inflammatory mediators were quantified, leukocytes and platelets counted and lung samples were collected for RT-PCR, immunohistochemical, and histological analysis. Female sex hormones and corticosterone were reduced 6 h after BD in non-OVx group. The infiltration of leukocytes in female non-OVx lungs was higher compared to OVx. G-CSF, VEGF, and CINC-1 were found increased in non-OVx group serum in comparison to OVx. Lung mediators were increased in non-OVx rats compared to controls. The acute reduction of sex hormones induced by BD appears to have a worse effect on lung inflammation than a reduction that has happened over a prolonged period of time, allowing a physiological adaptation prior to BD.

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Key words
brain death, female sex hormones, ovariectomy, rat, lung inflammation, organ donor

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Introduction

Lung transplant is a life-saving option for patients with lung failure. Over the past decades basic and clinical research have improved the outcome of lung transplantation, but the shortage of donors still is the main limitation. Due to a series of complications such as pulmonary contusion, aspiration, mechanical ventilation pressure injury, ventilator-associated pneumonia, and neurogenic pulmonary edema, the availability of lungs for donation is low in comparison to other solid organs.

In addition, the data available from organ transplantation show that donor's age negatively influences the graft survival of all organs, as well as increases the risk of early complications [1]. Aging is a process that occurs in both men and women however, the onset of menopause marks a sudden increase in the process of vascular aging. Brain death triggers multiple effects and affects hemodynamic control and hormonal milieu. The

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result is the worsening of organs' function and reduction of their viability.

At the early stages of an inflammatory reaction, endogenous glucocorticoids are secreted in large amounts and regulate the course of the response. In this regard, brain death (BD) is associated with a pronounced reduction in serum corticosterone level [2], that could contribute to the establishment of a systemic inflammatory process. Previous results from our group indicated that female rats present a higher lung inflammation status after BD that occurred in parallel with an acute reduction of the concentration of female sex hormones in the serum [3]. Indeed, some studies suggest a possible relationship between reduction on female sex hormone concentration and a proinflammatory state, especially in postmenopausal women, but the findings are conflicting [4–6]. Using trauma models, we and other authors have shown an increased lung inflammatory process in female animals submitted to ovariectomy [3,7]. Moreover, hormonal changes and their potential effects on inflammation may also explain the sharp increase in the risk of cardiovascular disease occurring in women after menopause [8].

Since sex hormones have been shown to modulate the inflammatory process after brain death and female lungs present higher inflammation in comparison to males, the goal of our study was to determine whether sex hormones modulate the lung inflammatory status observed after BD in female rats.

Materials and methods

Study groups

Animals were distributed in two groups: (i) female rats from high estradiol secretion (proestrus) to heat period (estrus) and (ii) ovariectomized (OVx) female rats. Vaginal smears' morphologic features were used in order to determine the estrous cycle. Rats from both groups were subjected to BD. In parallel, controls non-BD female rats, ovariectomized or not, were also included in this study.

Animals

Female Wistar rats (200–250 g) were housed in groups of three rats per cage (12-h light–dark cycle, 21 ± 2 Celsius) with free access to food and water. The experiments were approved by the local Animal Care Committee and followed the ARRIVE guidelines and the rules of the Brazilian Council of Animal Experimentation.

Ovariectomy

Animals 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 single doses of pentabiotic (570 mg/kg, i.m.) and tramadol (5 mg/kg, i.p). Animals received analgesic (paracetamol, 530 μg/ml) in the drinking water ad libitum for 3 days. Ovariectomy (OVx) effectiveness was confirmed after ten days by vaginal smears and uterine weight measuring.

Rat model of BD

Anesthesia was induced with 5% isoflurane and maintained with 2%. Animals were intubated and ventilated (10 ml/kg, 70 breaths/min, and FiO_2 100%). The skull was perforated, and a Fogarty- 4F catheter (Baxter Healthcare Co., Irvine, CA, USA) was inserted intracranially. The balloon was rapidly filled with 400 μl saline solution in order to increase intracranial pressure and induce BD, which was confirmed by maximally dilated and fixed pupils, apnea, absence of reflexes, and a drop in mean arterial pressure (MAP). The anesthesia was interrupted, and rats were maintained for 6 h under mechanical ventilation. Blood pressure was monitored and fluid was administrated (saline solution, 2 ml/h).

Quantification of serum levels of female sex hormones and corticosterone

Estradiol, progesterone, and corticosterone serum concentrations were determined using enzyme immunoassay kits according to the manufacturer's instructions (Cayman Chemical, Ann Arbor, MI, USA).

Cell counts in the bronchoalveolar lavage and white blood cells and platelet counts in the blood

Tracheas were cannulated with a polyethylene tube (1 mm inner diameter) and bronchoalveolar lavage (BAL) fluid was obtained by flushing with RPMI-1640 medium (10 ml). After centrifugation (170 *g* for 15 min at 15 °C), the resulting cell pellet was resuspended in PBS (1 ml). The total cell number in cell suspension (90 μl) stained with 10 μl of 0.2% crystal violet was determined in Neubauer chambers.

White blood cell and platelet counts were measured at baseline (0 min) and 6 h using an automatic

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hematology analyzer (Mindray BC 2800 Vet; Shenzhen, China).

Determination of inflammatory mediators in serum and lung explant samples

G-CSF, VEGF, CINC-1, MCP-1, IL-1beta, and IL-10 concentrations were quantified using Milliplex MAP kits (Darmstad, Germany) in samples of serum and supernatants of lung explants in culture [9]. Briefly, lung circulation was flushed through the right heart with 5 ml of PBS in order to remove the intravascular blood. Then, lung parenchyma samples were cut into small pieces (2 × 2 mm) and incubated in 24-well plastic plates (four pieces per well) with 1 ml of Dulbecco's Modified Eagle Media (37 °C and 5% CO_2). After 24 h, the media was stored in aliquots at 80 °C. The tissue pieces were dried in an oven at 60 °C and subsequently weighed. Results are expressed as pg of cytokine produced per mg of dry-weight of lung tissue.

Immunohistochemical analyses

After 6 h of BD, left lung was perfused with PBS and then expanded by intratracheal injection of 5 ml optimal cutting temperature media diluted in PBS (1:3). Hexane solution in nitrogen was used for fast freezing of the lungs. Cryosections (8 mm) were fixed in cold acetone for 10 min for further direct immunohistochemical assays as follows. PBS-BSA solution (2%) was used for blocking of nonspecific binding sites. Primary antibodies were anti-rat intercellular adhesion molecule 1 (ICAM-1) (1:50; Cedarlane, Burlington, ON, Canada), anti-rat vascular adhesion molecule (VCAM), and anti-iNOS (both 1:100; Abcam, Cambridge, MA, USA). The overnight incubation with primary antibodies was followed by horseradish peroxidase (HRP) secondary antibodies (Millipore, Burlington, MA, USA). Horseradish peroxidase substrate (3-amino-9-ethylcarbazole; Vector Laboratories, Burlingame, CA, USA) was used for staining, and the resultant stained areas were quantified using an image analyzer (NIS-elements; Nikon, Melville, NY, USA). In parallel, lung sections were incubated with an anti-rat CD49b FITC conjugated (1:100; BD Biosciences, San Jose, CA, USA) in order to stain the platelets. The perivascular platelets were quantified using an image analyzer coupled with a fluorescence microscope. The background reaction was determined in lung sections incubated in the absence of primary antibody (negative control).

Lung gene expression of iNOS

Gene expression by real-time PCR was quantified in lung tissue samples obtained 6 h after BD using a StepOne Plus Real-Time PCR System® (Applied Biosystems, Foster City, CA, USA). RNA extraction of the tissue was performed using a commercial kit (Mirvana®, Ambion®; Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. High-Capacity Reverse Transcriptase Kit (Applied Biosystems) was used for cDNA transcription. The primers used were all by TaqMan (Applied Biosystems): GAPDH (Rn01775763_g1), β-actin (Rn00667869_m1*), iNOS (Rn00561646_m1*) e endothelin-1 (Rn00561129_m1). The cycling conditions were 2 min at 50 °C, 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. The relative gene expression presented was determined in relation to control values obtained from samples of non-OVx or OVx rats not submitted to brain death ($n = 3$).

Statistical analysis

Data are presented as means ± SEMs. Comparisons between groups were made by two-way analysis of variance followed by Tukey's multiple comparisons test. All statistical analyses were performed with GRAPHPAD PRISM software, version 6.0, (GraphPad Software Inc., La Jolla, CA, USA).

Results

Female sex hormones and corticosterone concentrations after brain death

The decrease in estradiol and progesterone was evident in OVx control rats in comparison to non-OVx control rats. Similarly, BD non-OVx rats presented a significant reduction in sex hormones in comparison to non-OVx control rats. However, no differences were found after BD in OVx rats in comparison to respective control group (Fig. 1a,b).

Levels of corticosterone were reduced in OVx control rats in comparison to non-OVx control rats. Corticosterone was markedly reduced after BD, regardless of the removal of ovaries (Fig. 1c).

Effects of female sex hormones on leukocyte mobilization into lungs after brain death

There were no differences in white blood cell counts among groups (Fig. 2a). Conversely, BD induced a

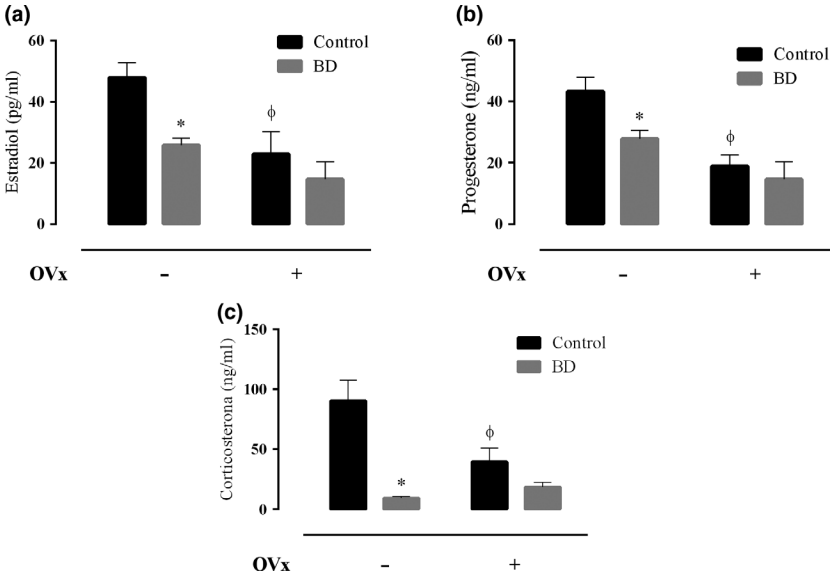

Figure 1 Serum concentrations of estradiol (a), progesterone (b), and corticosterone (c) 6 h after brain death. Groups consisted of female non-OVx and female OVx submitted or not (Control) to BD. Data are expressed as the mean ± SEM of six animals. $^{*}P < 0.05$ in comparison to respective control, $^{\Phi}P < 0.05$ in comparison to control non-OVx. BD, brain death; OVx, ovariectomized.

significant increase in bronchoalveolar (BAL) cell count in non-OVx rats that was not observed in OVx rats (Fig. 2b).

In order to evaluate cell infiltration in lung tissue, lung leukocytes numbers were analyzed by histology. There was higher number of leukocytes in the lungs of both groups submitted to BD in comparison to controls. Moreover, there were higher leukocyte counts in the lungs of BD non-OVx rats in comparison to BD OVx rats (Fig. 2c).

In parallel, platelet presence was analyzed in blood and in the lungs. Blood platelet counts decreased in both BD groups in comparison to controls (Fig. 3a), but no differences were found in the lungs among groups (Fig. 3b).

BD induced systemic and local release of inflammatory mediators

To investigate inflammatory components involved in systemic and lung inflammatory process after brain death, concentrations of G-CSF, VEGF, CINC-1, MCP-1, IL-10, and IL-1β were measured in blood and lung culture media. The results are shown in Table 1.

G-CSF, VEGF, and CINC-1 concentrations increased in serum after BD in non-OVx rats in comparison to controls and differed from BD OVx rats. As well as in serum, G-CSF and VEGF concentrations in lung explant increased after BD in non-OVx rats compared to non-OVx control group. On the contrary, CINC-1 concentrations in lung explant were found increased in OVx

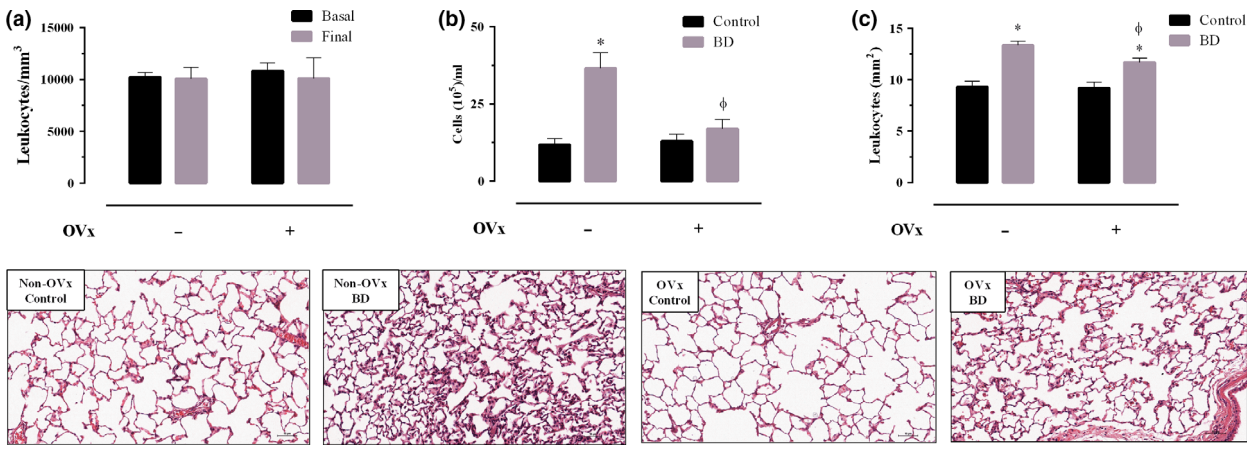

Figure 2 Effect of brain death on blood leukocytes counts (a), bronchoalveolar cell counts (b) and lung leukocyte infiltrate (c). Groups consisted of female non-OVx and female OVx submitted or not (control) to BD. (a) Data are expressed as the mean ± SEM of six animals. $^{*}P < 0.05$ in comparison to respective control, $^{\Phi}P < 0.05$ in comparison to BD non-OVx. Light photomicrographs shown hematoxylin and eosin-stained sections. BD, brain death; OVx, ovariectomized.

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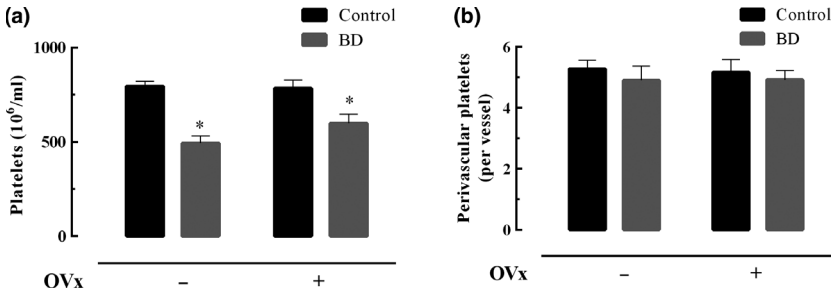

Figure 3 Effect of brain death on blood platelet counts (a) and on perivascular platelet number (b) in lung sections. Groups consisted of female non-OVx and female OVx submitted or not (Control) to BD. Data are expressed as the mean ± SEM of six animals. In (b) Platelets were marked with specific antibody by immunohistochemistry. One section per animal of five animals per group was analyzed. *$P < 0.05$ in comparison to respective control. BD, brain death; OVx, ovariectomized.

control group in comparison to non-OVx control and reduced after BD.

Serum MCP-1, IL-10 concentrations were higher in both groups submitted to BD compared to respective controls. In lung explant, MCP-1 and IL-1β concentrations were elevated only in the non-OVx group in comparison to control, whereas IL-10 was found higher in BD OVx group compared to its control group.

Female sex hormones influence on lung adhesion molecules and iNOS expression

Considering the inflammatory cells infiltration into the lungs, the evaluation of ICAM-1 and VCAM-1 expression is relevant. The results indicated ICAM-1 and VCAM-1 expressions decrease after BD in OVx group compared and non-OVx group (Fig. 4a,b).

Table 1. Serum (6 h after BD) and lung explant media (24 h incubation) concentrations of inflammatory mediators.

	Non-OVx		OVx	
	Control	BD	Control	BD
G-CSF				
Serum	3.55 ± 0.35	6.02 ± 0.37*	4.2 ± 0.63	3.78 ± 0.45**
Explant	0.029 ± 0.007	0.186 ± 0.04*	0.056 ± 0.011	0.115 ± 0.038
VEGF				
Serum	38.65 ± 14.4	89.86 ± 11.6*	38.11 ± 6.8	35.93 ± 14.5**
Explant	684.1 ± 162.4	2286.7 ± 466.9*	1105.9 ± 307.1	2014.4 ± 301.3
CINC-1				
Serum	65.6 ± 13.2	675.1 ± 120.2*	84.7 ± 18.9	320.7 ± 61.9**
Explant	1802.1 ± 613.5	888.4 ± 204.9	4951.2 ± 670.6***	1147.3 ± 210.5*
MCP-1				
Serum	461 ± 101.4	13874.2 ± 2665.3*	572.8 ± 53.6	13026.13 ± 2521.9*
Explant	448 ± 79.7	2131.8 ± 468.5*	1387.8 ± 189.1	1160.6 ± 246.8
IL-10				
Serum	43.9 ± 9.4	1012.6 ± 167.3*	39.2 ± 5.8	999.6 ± 223.4*
Explant	22.3 ± 7.2	21.2 ± 6.5	19.7 ± 1.4	68.6 ± 20.3
IL-1β				
Serum	19.5 ± 8.9	203.8 ± 59.4*	13.9 ± 6.3	191.6 ± 57.9*
Explant	8.9 ± 3	71.9 ± 11.4*	4.5 ± 0.9	47 ± 8.9

Groups consisted of female and female OVx submitted or not to BD. Concentrations were determined by ELISA. Data are expressed as mean ± SEM of six animals.

*$P < 0.05$ relative to respective control.

**$P < 0.05$ relative to non-OVx BD.

***$P < 0.05$ relative to non-OVx control.

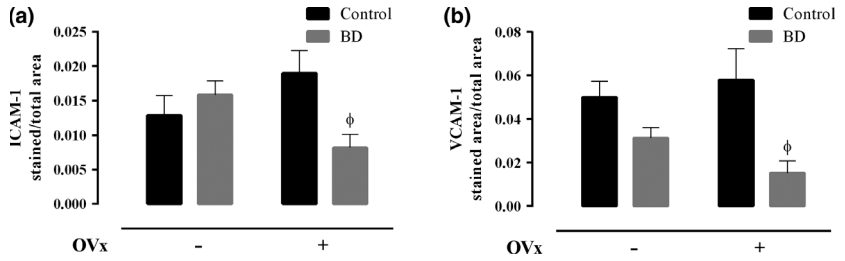

Figure 4 Lung expression of (a) intercellular adhesion molecule 1 (ICAM-1) and (b) vascular cell adhesion protein 1 (VCAM-1). Groups consisted of female non-OVx and female OVx submitted or not (control) to BD. The quantifications were determined by immunohistochemical analyses in lung sections, one section per animal of five animals per group. Data are expressed as the mean ± SEM. $^{\Phi}P < 0.05$ in comparison to BD non-OVx. BD, brain death; OVx, ovariectomized.

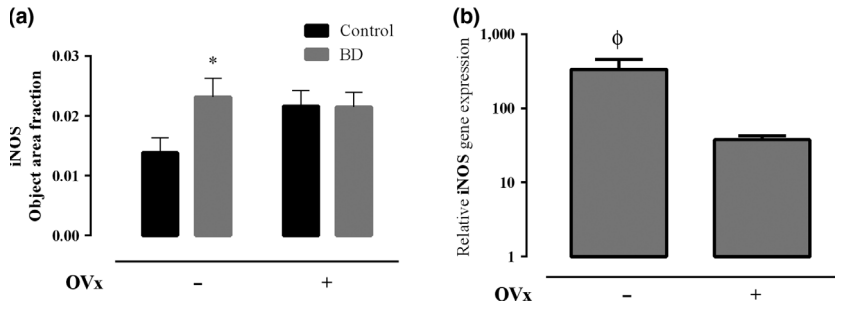

Figure 5 Effect of brain death on iNOS (inducible nitric oxide synthase) protein (a) and gene (b) expression. Groups consisted of female non-OVx and female OVx submitted or not (Control) to BD. In (a) quantification by immunohistochemical analyses in lung sections, one section per animal of five animals per group. In (b) Expression of iNOS in BD groups determined by RT-PCR in relation to expression of respective control groups. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ in comparison to respective control. $^{\Phi}P < 0.05$ in comparison to OVx BD. BD, brain death; OVx, ovariectomized.

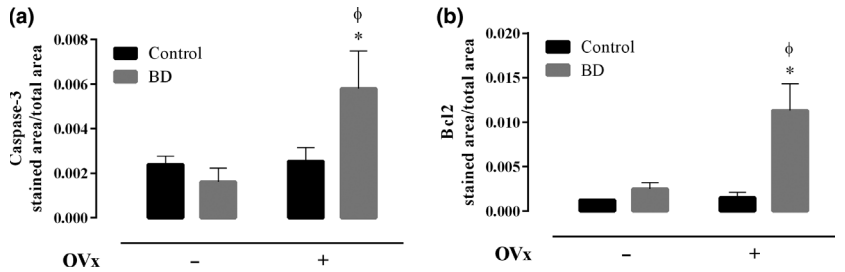

Figure 6 Effect of brain death on apoptosis-related proteins expression (a) caspase-3 and (b) Bcl-2. Groups consisted of female non-OVx and female OVx submitted or not (control) to BD. Quantification by immunohistochemical analyses in lung sections, one section per animal of five animals per group. Data are expressed as the mean ± SEM. $^{*}P < 0.05$ in comparison to respective control. $^{\Phi}P < 0.05$ in comparison to non-OVx BD. BD, brain death; OVx, ovariectomized.

In addition, lung iNOS protein and gene expression were evaluated as an inflammatory marker. It was found increased expression on both, iNOS protein and gene, in non-OVx rats after BD (Fig. 5a,b).

Lung caspase-3 and Bcl-2 expression: female sex hormones influence

Lung caspase-3 expression increased in BD OVx rats in comparison to all other groups (Fig. 6a).

Similarly, Bcl-2 expression in lung was increased after BD in OVx rats compared with other groups (Fig. 6b).

Discussion

Brain death results in the development of a systemic inflammatory response in the donors that is accompanied by an increased cell infiltration to the lungs [10]. In this study, we have compared the effect of brain death in female rats with no ovaries, therefore with very

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low sex hormone levels, to animals with normal hormonal levels. The response to trauma is reported to be worse in OVx rats when compared to hormonally active rats, suggesting that sex hormones are protective [35]. However, in our study, the lung inflammatory response after brain death was found higher in non-OVx rats in comparison to OVx animals, observation that was confirmed by the histological evaluation of lung tissue.

Female gonadal steroids play an excitatory role in corticosterone release, and it has been shown that ovariectomy leads to a reduction of corticosterone levels [11]. Basal corticosterone changes are linked to alterations in stress responsiveness [12] so it could potentially influence the response to brain death. Endogenous corticosteroids are effective in suppressing inflammation by reducing inflammatory cell numbers and reducing chemotactic mediators and adhesion molecules [13]. Moreover, with the reduction of serum estradiol levels, ovariectomy also leads to a decline of the expression of estradiol receptors [14] so this study can contribute to a better understanding of the status of the lungs of menopausal female donors. In this context, it is important to consider that, unlike in non-OVx rats, the hormonal reduction in OVx group occurred over a period of 10 days until the BD induction. Evidence has shown that after ovariectomy, there is a gradual decrease in uterine weight until the ninth day, indicating an adaption period [15]. In parallel, we also observed a reduction of corticosterone levels after OVx, characterizing an alteration of the hormonal milieu during this time. In contrast, in the non-OVx group, which presented higher inflammatory status in the lungs after BD, the reduction of female sex hormones and corticosterone occurred abruptly and with no adaptation period, suggesting that the rapid hormonal decrease observed in this group may have a negative impact on the inflammatory response induced by BD.

Despite the higher leukocyte infiltration into the lungs after BD in non-OVx rats, we did not observe differences in white blood cell counts among groups. The blood platelet number, however, was found decreased after BD in both groups. Platelets are recruited to inflammatory sites and contribute to host defense and cell recruitment. In pathological conditions, the extent of platelet deposition can contribute to occlusive thrombus formation and thrombocytopenia is reported to lead to increased permeability of systemic and pulmonary vessels in several models and to be associated with vascular leak in clinical conditions [16]. Although previous results showed higher microvascular permeability in female animals after BD [17], the analysis of platelets presence in the lung tissue did not show differences among groups. However, study from Cleary *et al.* [18] showed adhesion of platelets in the lung microvasculature in an inflammatory response induced by LPS that was not associated with thrombus formation, occurring through mechanisms distinct from those mediating neutrophil recruitment or the occurrence of pulmonary emboli. Since we did not analyze the intravascular compartment, further experiment is necessary to confirm if this could be the case in BD as well.

In order to identify some of the mediators involved in the lung inflammatory process after brain death, we quantified growth factors, chemokines, and cytokines in serum and in lung tissue culture media. After BD, there is an increase of serum G-CSF, that is higher in non-OVx group. The induction of G-CSF production by inflammatory cytokines mainly produced by innate immune cells is a key mechanism eliciting neutrophil production [19], and its local release is found increased in the lungs after a systemic inflammatory model as hemorrhagic shock [20]. G-CSF enhances neutrophils maturation process, survival, and function including cytokine production [21]. There was also higher G-CSF release in the lungs of non-OVx rats after BD, confirming a higher inflammatory activity in these animals.

Serum VEGF concentrations were found elevated in BD non-OVx group which could enhance the systemic inflammatory status by increasing vascular permeability [22]. More importantly, VEGF was highly increased in the lung tissue, which could favor leakage of plasma proteins and inflammatory cells into the extravascular space compartment. In parallel to VEGF, we analyzed the chemokine CINC-1, that is mentioned as the rat homolog of human IL-8 [23]. The results showed elevated serum CINC-1 concentration in BD animals, higher in non-OVx group. In contrast, we found higher levels of CINC-1 released by the lungs of OVx control group, which could be an effect of a low grade of systemic inflammation that is characteristic after ovariectomy [24]. MCP-1, another important chemokine with specificity for monocytes/macrophages, was analyzed and the results pointed to higher serum concentrations after BD and a significantly increased lung release in BD non-OVx in comparison to controls. Estradiol at physiological concentrations inhibits MCP-1 expression *in vivo* [25] and the increased concentrations found after BD could be resultant of the estradiol acute reduction in non-OVx BD rats and might contribute to the lung inflammatory process establishment.

As a result of activation of inflammation after BD, there is a local and systemic release of inflammatory cytokines like IL-1beta and IL-10 [26,27]. Our findings confirm the systemic release of IL-1beta and IL-10 after BD. The lung release of the cytokine IL-10, however, did not increase in

BD non-OVx group as observed in BD OVx group ($P = 0.054$). The lack of increase in IL-10 levels in the lung tissue could corroborate to higher lung inflammatory status found in this group, once IL-10 plays important role on the inhibition of the inflammatory end-points and in the suppression of functions of monocytes/macrophages in both innate and specific immunity [28].

Considering that our data evidenced higher cellular infiltrate after BD, we analyzed the expression of endothelial adhesion molecules, ICAM-1 and VCAM in the lung tissue. The results showed lower expression of both molecules in the lungs of the BD OVx group in comparison to BD non-OVx. Lichte *et al.* [29] showed that IL-10 affects the local ICAM-1 expression, reducing the expression in the lung. Even though the levels of IL-10 in lung tissue were much lower in comparison to systemic levels, we observed a 3-fold increase of this cytokine after BD in OVx rats. It is possible to speculate that this increase is reducing the expression of these adhesion molecules in the lung of OVx rats therefore, contributing to the lower number of leukocytes recruited to the lung observed in this group.

After activation of inflammatory process in the lungs, alveolar macrophages, and neutrophils express iNOS and produce large amounts of NO, thus, resulting in tissue injury [30]. Previous work from our group reported that iNOS accumulated significantly in inflammatory cells in the lung parenchyma after brain death in male rats [31]. Here, we showed that BD non-OVx rats presented higher iNOS protein expression in comparison to control. The OVx groups, however, showed a constitutively higher but not significant expression of iNOS that was not altered by BD, which seemed to confirm the before mentioned low grade of systemic inflammation that is characteristic after ovariectomy [24]. In addition, the iNOS gene expression was also increased in the lungs of BD non-OVx group in comparison to BD OVx, which indicate a post-transcriptional influence of the female sex hormones.

Brain death induces activation of the apoptosis cascade in the donor organ and the induction of cell death occurs both by activation of the cell-surface death-receptor-mediated pathway and the mitochondrial pathway [32]. Apoptosis is a physiologic process regulated by a specific pathway, which can lead to programmed cell death and is influenced by estradiol [33]. Caspase-3 is a proapoptotic protein, the terminal executioner protease in apoptosis, where it initiates dismantling of cellular components via cleavage of structural proteins, disruption of the nuclear envelope, and breakdown of genomic DNA. On the other hand, BCL-2 protein family is formed by the prosurvival cell guardians, which render many cell types resistant to diverse apoptotic stimuli. The higher lung expression of caspase-3 and BCL-2 in BD OVx rats compared to other groups points to an important estradiol regulation on cell death after BD. The increase in lung apoptotic markers such as caspase-3 due to estrogen deficiency has been previously described in aging female mice that was accompanied by increased number of apoptotic cells in the lung [34].

Taken together, these findings contribute to a better understanding of the influence of female sex hormones on the lung repercussions observed after brain death. Females with normal hormonal concentrations at the time of the BD induction presented an intense lung inflammatory response with cell infiltration and release of inflammatory mediators. On the other hand, ovariectomized females submitted to brain death presented a lower lung inflammatory response which could be a result of a physiological adjustment to the lack of sex hormones, impacting the immune system as a whole. Thus, the quality of the lung of a female donor could be highly affected by her hormonal status. It is our view that the effect of sex hormones on the lung quality should be subject of further analysis on the graft survival in the receptors.

Authorship

ALBA: performed the study, analyzed data and wrote the manuscript. CJC, RAJr, FYRS and MVS: contributed obtaining and analyzing data. SGF: contributed obtaining the data. ACBF: designed the study, analyzed data and wrote the manuscript. YRV and LFPM contributed analyzing data and wrote the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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