Sexual Dimorphism of Elastic Fibers in Prenatal Lung Mice

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ABSTRACT

Scientific data has revealed the existence of lung gender differences and therefore sparked a renewed interest in understanding the underlying mechanisms and their effect in the healthy lung development and/or in pathological conditions. Elastic fibers have an important role in lung development during pre and post-natal stages, because a well-developed pulmonary elastic fibers favour pre-natal lung maturation and enhance alveolarization. Sexual differences studies on lung elastic fibers content are focused essentially on the post-natal stage, with scarce data on pre-natal lung development. Using an experimental mice model, we developed this research work to study gender differences in the lungs elastic fibers during gestational days E15-E19, using image analysis and elastin HPLC methodologies. Our results show significant sexual dimorphism in lung elastin and elastic fibers content pre-natal stage, which is more evident in the last two gestational days (E18 and E19). Female’s mice have more elastin and elastic fibers which could mean that the elastogenesis process begins earlier than males. These results are an important contributes to understand the underlying factors involved in physiology and lung development sexual difference.

Keywords: Elastic fibers, Fetal lung, Lung development, Sexual dimorphism.

1. INTRODUCTION

Clinical epidemiologic data supports the gender differences morbidity, and mortality incidence in airway diseases including asthma (Kynyk et al., 2011; Zein & Erzurum, 2015), chronic obstructive pulmonary disease (COPD) (Laviolette et al., 2007; Barnes, 2016), cystic fibrosis (CF) (Saint-Criq & Harvey, 2014; Sweezy & Ratjen, 2014), lung cancer (Zang & Wynder, 1996; Stabile & Siegfried, 2003) and non-CF-related bronchiectasis (Morrissey & Harper, 2004; Vidaillac et al., 2018).

Gender differences have also been observed in preterm newborns, infants that have major morbidity and mortality than infants born at term evidencing the key factor of gestational age in the development of neonate diseases (Kent et al., 2012; Zisk et al., 2011; Shim et al., 2017). The undeveloped lungs of preterm males have higher incidence of respiratory distress syndrome, pneumothorax, early respiratory support and subsequent bronchopulmonary displayia or chronic lung disease (Townsel et al., 2017; Silveyra et al., 2021). The high mortality in preterm males can be largely attributed to increased acute respiratory disorders, which is due in part, to the male delayed lung maturation (Khoury et al., 1985; Merkus et al., 1996).

Despite the antenatal corticosteroid administration and postnatal surfactant clinical use, there is a gender gap in neonatal outcomes, with a higher rate of morbidity and mortality in males (Stevenson et al., 2000; O’Driscoll et al., 2018; Townsel et al., 2017; Silveyra et al., 2021).

In rats and mice sexual dimorphism is already observed in the alveolar architecture, more visible at the time of sexual maturity (Massaro et al., 1995, 1996; Massaro & Massaro, 2004, 2006; Patrone et al., 2003). Females have more alveoli, larger alveolar surface and smaller alveoli when compared to males, despite the fact that both have the same oxygen consumption rate (Massaro et al., 1993, 1996; Massaro & Massaro, 2006).

Elastic fibers play an important role during pre- and post-natal lung development participating in the septal formation that occurs during the alveolization process and thus contributing to normal adult lung physiology. This active role has been suggested by the temporal association between the appearance of elastic fibers bundles at the secondary septa tips and new alveoli formation (Fukuda...
et al., 1984; Bruce & Honaker, 1998; Mariani et al., 1997). In rats and mice, the tropoelastin (TE) expression initiates in the pseudoglandular stage and is detected in bronchi and lung arteries and is almost undetected in pulmonary proximal mesenchyma (Mariani et al., 1997; Mariani & Pierce, 1999). In the canalicular stage TE begins to appear in distal parenchyma, specially located around the epithelial branched structures. In the sacular stage elastic fibers are found near terminal sacs (Pierce et al., 1995; Mariani et al., 1997) and during alveolization high content of TE appears in the alveolar wall, where is needed for normal alveolization process (Mariani et al., 1997).

Factors that disturb the elastin synthesis or assembly during prenatal period, will lead to abnormal lung development. Prenatal undeveloped elastogenic process will affect distal branching and alveolarization and consequently compromise lung maturation, resulting in permanent lifetime defects (Buckingham et al., 1981; Rucker & Dubick, 1984, Wendel et al., 2000; Nakamura et al., 2002; Mecham, 2018).

Lung development sexual disparity will have a multifactorial explanation but so far, the underlying mechanisms are not fully understood. Differences in the respiratory anatomy and physiology, immune response, genetic predisposition, hormonal and environmental aspects must be some of the factors involved in this gender dichotomy (Pinkerton et al., 2015; Raghavan & Jain, 2016; Chamekh et al., 2017; Tam et al., 2011; Carey et al., 2007b; Merkus et al., 1996). And since elastic fibers play an important role during structural lung development, it is important to understand if there is elastogenic lung gender dichotomy. Therefore, the aim of this experimental work is to quantify and compare male and female lung elastic fibers during the prenatal period, data is currently lacking. A quantitative image analysis and HPLC elastin quantification from the prenatal period, sex identification.

The lung samples collected for histology image analysis were fixed in formaldehyde solution (4%; pH 7.4) and the samples for HPLC elastin quantification were frozen at −80 °C.

2.1. Elastic Fibers Image Analysis

We collect the lungs of seven mice fetuses in each gestational day, always belonging to at least three different litters. The lung samples for light microscopy analysis were processed and cut with 4 μm thickness and their slides stained with Gomori modify technique development in our histology institute to quantify the elastic fibers (Gonçalves et al., 2001).

The lung lobules of each group/gestational day were photographed entirely using Nikon microscope, an Olympus photographic system and the Studio Lite® 1.0 photo program. Images were captured with 400× magnifications and saved in 16 million tiff formats. For more accuracy, we select to evaluate the images with less blood vessels and less conducting structures, and in some cases eliminate erythrocytes in the blood vessels or any dye waste present in the image using Adobe Photoshop program.

In each group we quantify six images per mice and eliminate the highest and lowest value, giving a total of 28 determinations per group/per gestational day, using the ImageJ® 1.36 program. The total septal area was determined by the program and corresponds to the total amount of pixels present in the 8-bitt image. To determine the elastic fibers present in the respective image we convert the initial 16 million tiff image (Fig. 1a) into an 8-bitt image (Fig.1b). Then we activated and adjusted the threshold (Fig. 1c) and obtaining a two-tone image (Fig.1d), which allowed the quantification of the pixels of the dark particles, which corresponds to the elastic fibers. With these two previous results we determined the percentage of elastic fibers per septal area (Bolender et al., 1993; Gonçalves et al., 2001).

To analysed septal area and elastic fibers development during gestational days E15–E19, we compared the data of the three development stages included in this experiment. The E15 lungs stand for pseudoglandular, E17 to canicular and E19 to sacular stages and in each gender, we will compared E15 to E17 and E17 to E19.

2.2. HPLC Studies

The HPLC methodology was previous used and proved to be reliable for quantitative analysis elastin synthesis (Rodrigues et al., 2008; Gonçalves et al., 2001).

2.3. Desmosine and Isodesmosine Quantification

The number of lung samples collected for this study depends on gestational day due to the lung size, but the total sample weight was similar for each day in both groups. The Table I resumes the number of embryos used in each gestational day and the number of litters used to obtain each gestational sample per group. We collected the entire lung from each embryo but eliminate trachea and extrapulmonary bronchi.
The method to quantify lung elastin comprises HPLC determination of desmosine and isodesmosine and spectrophotometry quantification of total protein present in the sample, and the final result will be expressed in pmol per μg of protein. HPLC quantifications were done in the pool of lungs from each group and gestational day, making four determinations per group/gestational day.

The extraction and quantification process were done using methodology previously developed in our laboratory with minor adjustments related with the biological material (Rodrigues et al., 2008).

2.3.1. Elastin Extraction and Hydrolysis

After being weighed, the lungs were collected, frozen (−80 °C), lyophilised, and dried under vacuum (+40 °C). The samples were homogenized (in 2 ml of water), and half of the homogenate was frozen (−20 °C) for total protein quantification. The remaining 1 ml was mixed with 1 ml water and 2 ml of trichloroacetic acid (TCA) solution (10%) and centrifuged for 10 minutes (9000 g at +4 °C). The supernatant was discarded, and the residue was treated with 5 ml of 5% TCA (at +90 °C) for 30 minutes to extract the collagen (Fitch et al., 1955). The sample was centrifuged for 10 minutes (9000 g at +4 °C), and the supernatant was discarded. The TCA extracted residue was washed twice in 2.5 ml acetone, followed by centrifugation (9000 g). The residue was dried under vacuum (+60 °C), and this final product constitutes the lung sample that will advance for elastin hydrolysis.

The hydrolysis process will be performed on our lung samples, 2 mg elastin solution, and a standard desmosine and isodesmosine, were kept in the stove (60 °C–65 °C) until the purification process began.

Reagents used in this process: Trichloroacetic acid (Merck®); Acetone (Riedel-de Haen®); Elastin (EPC-elastin products Co, Inc; Ref E60); Desmosine and isodesmosine (EPC-elastin products Co, Inc; MD687); Hydrochloric acid (BDH® Chemicals).

2.3.2. Desmosines Purification by a Cellulose Mini-Column

The desmosine isolation in an almost pure form was attained by using a small column of cellulose (Skinner, 1982). To prepare the mini-columns, we used disposable plastic Pasteur pipettes in which we cut off the top hemisphere of the bulb, becoming a 4 ml solvent reservoir. We prepare two “solutions” for this procedure: a mobile phase (n-butanol: acetic acid: water in a 4:1:1 proportion) and a stationary phase (a mixture of 1 g cellulose with 20 ml of the mobile phase). The tips of the pipettes were plugged with a small quantity of fibreglass and filled with 4 cm of stationary phase and 5 ml of mobile phase.

All previous hydrolyzed samples were maintained added, acetic acid (0.5 ml), stationary phase (0.5 ml), and n-butanol (2 ml). Purification begins by adding 2 ml of mobile phase in the columns, followed by the sample content and another 1.5 ml of mobile phase. Then more 15 ml of mobile was added, and all this elute filtrate was desipied. The columns were eluted with 5 ml of ultrapure water, and this filtrate was collected into test tubes. The aqueous fraction containing the desmosines was lyophilised before analysis (during 16 hours) to remove residual butanol and acetic acid and to concentrate the sample.

Reagents used in this process are n-butanol (Merck®) and acetic acid glacial (Panreac®).

2.3.3. Pre-Column Derivatization of Desmosines

This procedure was carried out according to Guida and colleagues’ method but with a slight modification (Guida et al., 1990). All the previous lyophilised samples were dissolved in 1 ml of hydrochloric acid, 0.01 M. We withdrew 100 μl from our samples and added 100 μl of sodium hydrogen carbonate 0.5 M and 100 μl of Dansyl (DNS) chloride 20 mM (in acetone). After 40 minutes in the dark (at +65 °C) and cool out at room temperature, the derivatization mixture was diluted in 3 ml of mobile phase A and mobile phase B (proportion of 70%/30%). Mobile phase A is 85:15 v/v of c 25 mM and acetic acid 25 mM, and mobile phase B is 40:60 v/v of the same reagents. The mobile phases were degasified, and the pH

| TABLE I: NUMBER OF EMBRYOS AND LITTERS PER GROUP IN EACH GESTATIONAL DAY |
|--------------------------|----------------|----------------|----------------|----------------|----------------|
| Gestational day          | E15 | E16 | E17 | E18 | E19 |
| No. of embryo            | 50  | 26  | 15  | 10  | 10  |
| No. of litters           | 15  | 9   | 6   | 5   | 5   |

Fig. 1. Image quantification of elastic fibers: a) tiff 16 millions image; b) 8-bitt image; c) image before threshold application; d) image after threshold application.
adjusted to 7.2. The same derivatization procedure was carried out for the working standard solution. Volumes of 100 μl of derivatization mixtures were injected into the chromatograph.

Reagents used in this process: Acetone (Riedel-de Haen®); Hydrochloric acid (BDH® Chemicals); Sodium hydrogen carbonate (Merck®); Dansyl (DNS) chloride (Sigma® Chemical).

2.3.4. Chromatographic Separations by HPLC

Was performed using a programmable liquid chromatographic system “Gilson, Unipoint, V1.9 system software” and the UVNVis detector Gilson 151 equipment. The readings were done with a wavelength of 254 nm and with sensitivity adjusted for 0.001 aufs. The HPLC column was a reversed-phase “Waters-Spherosorb, ODS2” stainless steel column (25 cm × 4.6 mm I.D.) from Waters associated, Inc. Milford, MA, USA. The flow rate was always 1.2 mL/minute, and the system was adjusted to elute desmosine at 20 minutes.

Desmosine and isodesmosine concentrations were determined by reverse-phase HPLC with a binary gradient elution of 30%–60% mobile phase (composition previously described). Between the sample analyses, the system was calibrated for 10 minutes with a gradient of 70% phase A and 30% B. The detector was adjusted to read at 254 nm, and quantification was performed by external calibration. For each sample and standard solutions, we made 4 analyses with the injection of 100 μl in HPLC loop. The samples from each gender and equal gestational day were analysed in the same day and injected in the HPLC equipment alternately.

The calibration curve was constructed by analysis of standard solutions containing scalar amounts of desmosines from 50 pmol/mL, 100 pmol/mL and 250 pmol/mL (Guida et al., 1990).

2.3.5. Total Protein Determination

For total protein quantification, we use a Micro-Lowry Peterson’s Kit (TP 0300) and based on the Lowry’s reaction, we analyse our samples and establish the calibration curve, with the final protein concentrations of 50, 100, 200, 300, and 400 μg/mL.

The 1 ml of the initial samples (only homogenised and maintain at −20 °C) were diluted in 1 ml distilled water, homogenized, and added 0.1 mL of sodium deoxycholate (0.15%) for 10 minutes. We added 0.1 mL of trichloroacetic acid (70% p/v) and centrifuged for 8 minutes (10000 rpm). The supernatant was neglected, and the “pellets” were dissolved in 0.1 mL of Lowry reagent (for 20 minutes) and then added Folin & Fenol Ciocalteu to give colour to the samples (for 30 minutes). All these procedures were done at room temperature. The absorbance was determined using the spectrophotometer (three times each sample), in a wavelength of 750 nm, and using the calibration curve established in the beginning of this methodology.

2.4. Statistical Analysis

All results were analysed with the program StatView 5.0. The statistical comparisons were made using the student-t test (paired), and the significant value was established at P ≤ 0.0001.

3. Results

3.1. Elastic Fibers Image Analysis

During the gestational days E15 to E19, we observed a progressive decrease in the septal area in the male group, trend that is also observed in females with the exception of day E17, where the value is slightly higher than day E16 (Table II). In both sexes the largest septal area decrease occurs between day E15 and E16 (statistically significant P ≤ 0.0001). In males, no other statistically significant results are observed along the gestation, but in females, we observe another statistically significant decrease between days E17 and E18 (P ≤ 0.0001).

The sex comparison of the septal area did not show statistically significant differences between the sexes. Nevertheless, females have lower septal areas than males, with the exception of day E17.

The septal area analysis during lung development stages evidences a reduction in both sexes (Fig. 2). In females and males, the biggest septal decrease occurs between pseudoglandular (E15) and canalicular (E17) stages (statistically significant P ≤ 0.0001 for both sexes), with an average reduction of almost one-third of the total area. Between the canalicular and sacular (E19) stages, the average septal decrease is similar in both, and almost half of the septal tissue is reduced (statistically significant P ≤ 0.0001 for both sexes).

The elastic fibers percentage per septal area study showed an increasing tendency in both sexes during gestational days E15–E19 (Table III). Nevertheless, these increases are only statistically significant between successive days E16 and E17 in both genders (statistically significant P ≤ 0.0001 for both sexes). The sex comparative analysis showed no statistically significant differences in elastic fibers percentage per septal area.

The elastic fibers per septal area analysis during lung development stages, evidence a statistically significant increase between pseudoglandular (E15) and canalicular (E17) stages in both sexes (P ≤ 0.0001). The transition between canalicular and sacular (E19) stages also exhibited an increase in both sexes, but not statistically significant (P ≥ 0.0001) (Figs. 3 and 4).

Fig. 2. Lung septal area in both sexes during pseudoglandular (E15), canalicular (E17) and sacular (E19) stages.
TABLE II: Lungs Septal Area from day E15 to E19 (Mean Value ± Standard Error)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day E15</th>
<th>Day E16</th>
<th>Day E17</th>
<th>Day E18</th>
<th>Day E19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>1573.8 ± 156</td>
<td>589.8 ± 52</td>
<td>619.4 ± 27</td>
<td>379.7 ± 22</td>
<td>315.4 ± 22</td>
</tr>
<tr>
<td>Male</td>
<td>1511.2 ± 61</td>
<td>631.1 ± 36</td>
<td>556.6 ± 28</td>
<td>443.5 ± 35</td>
<td>359.5 ± 17</td>
</tr>
</tbody>
</table>

Fig. 3. Histological image of elastic fibers in females (a) and males (b) lungs in day E19; Gomori modified technique, 200×.

TABLE III: Lungs Elastic Fibers % per Septal Area from day E15 to E19 (Mean Value ± Standard Error)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day E15</th>
<th>Day E16</th>
<th>Day E17</th>
<th>Day E18</th>
<th>Day E19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>4.02 ± 0.46</td>
<td>5.23 ± 0.45</td>
<td>10.22 ± 0.36</td>
<td>11.11 ± 0.68</td>
<td>13.2 ± 0.78</td>
</tr>
<tr>
<td>Male</td>
<td>4.18 ± 0.32</td>
<td>4.41 ± 0.21</td>
<td>10.54 ± 0.55</td>
<td>10.67 ± 0.71</td>
<td>11.5 ± 1.2</td>
</tr>
</tbody>
</table>

3.2. HPLC Quantification Analysis

The elastin content value was indirectly determined by the quantification of desmosine, and we observed that during gestational days E15 to E19, both sexes had a tendency to increase the amount of elastin synthesis (Table IV). Males experiment with small and steadier increases in desmosine content from day E15 to E19, while females exhibit significant daily increases with the exception of day E19. In males, the desmosine content differences are only statistically significant between days E16 and E17 (statistically significant P ≤ 0.0001). Females showed a distinct behavior, reaching a large peak on day E18 and decreasing on day E19. The statistical analyses of female results are statistically significant between days E17/E18 and E18/E19 (statistically significant P ≤ 0.0001).

The sex comparison only evidences statistically significant differences in days E18 and E19 (statistically significant P ≤ 0.0001). Female lung desmosine content is much higher than that of males, with a threefold value on day E18 and a twofold value on day E19 (Table IV).

The analysis along the lung development stages evidences a statistically significant increase in the amount of desmosine from the canalicular (E17) to sacular (E19) stage in both sexes (statistically significant P ≤ 0.0001). The transition between pseudoglandular (E15) to canalicular (E17) phases exhibited a small increase in both sexes, but these results were not statistically significant (P ≥ 0.0001) (Fig. 5).
4. Discussion

Gender dichotomy is a pertinent subject in several research areas, especially in lung development and in the clinical progress of some respiratory diseases (Townsel et al., 2017; O’Driscoll et al., 2018; Carvalho & Maina, 2019; Carvalho, 2020). During prenatal development, human male fetuses have more alveoli and alveolar surface area than age-matched females (Carey et al., 2007a). In rats and mice, the sex difference in the alveolar architecture is more visible during the sexual maturity phase (Massaro et al., 1995; Patrone et al., 2003; Massaro & Massaro, 2004, 2006). Female have more alveoli, larger alveolar surface and smaller alveoli when compare with males (Massaro et al., 1995, 1996; Massaro & Massaro, 2006).

Surfactant production appears earlier in female lungs (Fleisher et al., 1985), and the surfactant presence seems to prevent the early closure of alveoli and small airways, contributing to a higher airflow in the female respiratory system (Townsel et al., 2017). In fact, earlier surfactant production is mediated by female sex hormones (Trotter et al., 2000), and lung development maturation is stimulated by estrogens (Massaro & Massaro, 2006).

Female mice with deletion of estrogens receptor beta (ERβ) exhibit significant disturbance in lung development, with enlarged alveolar size and reduced alveolar surface area, making their lungs similar to male counterparts (Townsel et al., 2017). The biological effects of estrogens occur through the activation of two estrogens receptors (ERα and ERβ) that have a highly homologous structure but non-identical action and tissue distribution. In fact, gender differences were detected in the expression of estrogens receptors during prenatal lung development between gestational days E15 to E19 (Carvalho & Gonçalves, 2012).

The gender differences observed in preterm neonates can affect the development of respiratory disease. Preterm males have lower surfactant production and reduced gas exchange areas which strongly influence the risk of developing RDS (Townsel et al., 2017). The high incidence of BPD in males is not fully understood, but could be related with gender dimorphism in surfactant synthesis, that could be a deleterious effect of androgens in male fetus lung development (Seaborn et al., 2018; Dammann et al., 2000). When neonatal mice are exposed to hyperoxia, males have more lung inflammation; impair alveolarization and pulmonary angiogenesis, and higher mortality rate when compared with the opposite sex (Lingappan et al., 2016). These differences could explain the increased incidence of BPD in male premature neonates (Lingappan et al., 2016).

Our experimental research is focused in evaluating mice sex differences in the elastogenesis of prenatal lung because elastic fibers are essential for respiratory physiology and lung’s morphogenesis (Fukuda et al., 1984, Rucker & Dubick, 1984, Noguchi et al., 1989; Noguchi et al., 1990). In fact, the complete disruption of tropoelastin gene expression causes a defective lung development, with distal branching defects and emphysematous-like morphology, and a rate of 100% postnatal mortality (Wendel et al., 2000).

During gestational days E15–E19, we observed a progressive decrease in the septal area of the male’s lungs and similar results for females, with the exception of day E17, which exhibited a small increase. Despite small differences during the experiment, no statistical differences were detected between genders.

In both sexes, the highest decrease occurs between days E15 and E16, with a reduction of half the septal area. The gestational days E15 and E16 belong to pseudoglandular stage, a period in which lung conducting portions are differentiating and mesenchymal tissue remains thick (Ten Have-Opbroek, 1981, 1991). This sharp decrease that we observed on day E16 could be explained by the transition to the next development stage (canalicular), where lung air passages continue to develop and the mesenchymal tissue rearrangements continue to become thinner (Ten Have-Opbroek, 1981, 1991).

In both sexes, the percentage of elastic fibers per septal area increases along the analysed period, and the biggest rise occurs between days E16–E17, precisely the transition between pseudoglandular and canalicular stages. In females, the elastic fibers increase following the lung development stages, but in males, we did not observe a significant difference between the canalicular and sacular stages. Besides no statistically significant differences between sexes, these results may indicate that females initiate the sacular stage earlier than males.

Overall, our image analysis showed progressive septal area reduction and elastic fibers increase facts that are compatible with the continuous mesenchymal rearrangement and the elastogenesis process that occurs along lung development (Ten Have-Opbroek, 1981, 1991). These results are similar to another research work, developed in the same gestational days but with no sex separation (Rodrigues et al., 2008).

Our HPLC data supports the image analysis results and clearly highlight sexual differences in the lung elastogenesis process. Males have small elastin increases from day E15 to E19, while females have steep increases after day E16, reaching a peak on day E18. On days E18 and E19, females have elastin content threefold and twofold of the value detected in males (P ≤ 0.0001), and these results clearly show the existence of sex differences along prenatal lung development.

Our study evidences sex dimorphism in the developing of the canalicular stage, with males initiating this stage later than females and, for this reason, having less elastic fibers and elastin than females. We can consider that mesenchymal rearrangements and pre-alveolar sacs formation that occurs between canalicular and sacular stages are delayed in males. This study showed that females produce more elastin and that the elastogenesis process begins previous than males. The similarities between mice and human lung development could allow us to consider that this dimorphism is present in human fetus lungs.

The significant sexual differences that we observe occur at the end of gestation, the fact that is similar to some previous reports in other species (Davidson et al., 1984; Schellenberg et al., 1987; Pierce et al., 1995), that relate both biochemical quantification (Schellenberg et al., 1987) and in situ hybridization to tropoelastin mRNA (Davidson et al., 1984; Pierce et al., 1995). Several works of Massaro et al. (1995, 1996); Massaro and Massaro (2004, 2006)
have already shown postnatal gender differences in rats and mice more evident during the sexual maturity period.

The presence of more elastin and elastic fibers in female prenatal lungs could be important in the lung development process. This apparent male “disadvantage” remains relatively undetermined but could be explained by several factors such as genetic inherent, immunological, physiological differences, and steroid hormones. In fact, we have previously described the existence of sex dimorphism of estrogens receptors expression (Carvalho & Gonçalves, 2012). And since female deletion of estrogens receptor ERβ enhance alveolar size and reduce alveolar surface area, making female lungs look like their male counterparts (Townsel et al., 2017), estrogens receptors expression disparity could be one of the factors involved in the sex differences observed in this work.

5. CONCLUSION

In summary, sexual dimorphism should be considered for future lung experimental research and it is important to determine the factors involved in these differences. Sex disparity should be considered in future preterm neonate’s treatments or in uterus therapies adjusted for each gender.

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CONFICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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