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Oligohydramnios compromises lung cells size and interferes with epithelial-endothelial development

Tanbir Najrana, Ph.D[1].; Lauren M. Ramos, MD[1]; Rasha Abu Eid, PhD[2], Juan Sanchez-Esteban, MD[1*]

[1] Department of Pediatrics, Alpert Medical School of Brown University. Women & Infants Hospital of Rhode Island, 101 Dudley Street. Providence, RI 02905. USA[1]

[2] Dental School, University of Aberdeen, Aberdeen, AB25 2ZA, Scotland, UK.

** Corresponding author:* Juan Sanchez-Esteban, M.D. Department of Pediatrics. Women & Infants Hospital of Rhode Island. 101 Dudley Street. Providence, RI 02905. USA. Phone: 401-2741122, ext 47483. Fax: 401-4537571. E-mail: jsanchezesteban@wihri.org

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Keywords: Lung development; lung hypoplasia; alveolar type I epithelial cells; mouse model

Running title: **Oligohydramnios and lung development**

Abstract

Background and Objective

Severe oligohydramnios can induce pulmonary hypoplasia. However, the mechanisms by which leaking of fluids cause lung hypoplasia are not well defined. The objective of this study was to characterize a mouse model of pulmonary hypoplasia induced by oligohydramnios.

Methods

Amniotic sacs were punctured on E14.5 of gestation. Untouched fetuses were used as control. Pregnancy was allowed to continue until E18.5 in which lung tissue was collected and evaluated for morphometry, proliferation, differentiation, apoptosis and angiogenesis.

Results

Our results found that lung weight, lung to total body weight ratio, and lung water content were reduced in oligohydramnios when compared to controls. In contrast, oligohydramnios did not affect the DNA content. Morphometric studies confirmed that oligohydramnios fetuses had smaller air spaces than control. Interestingly, cells from oligohydramnios fetuses have smaller size and less regular shapes. Oligohydramnios decreased the differentiation of type I epithelial cells and compromised apoptosis and angiogenesis while proliferation was not affected.

Conclusions

Although the smaller size of the lung could be explained by a decreased of lung fluids, our data suggest that increased of external compression secondary to severe oligohydramnios can compromise cell size and interfere with epithelial and endothelial development. Type I epithelial cells could have an unrecognized key role in the differentiation of the distal lung mediated by mechanical signals.

Introduction

The importance of mechanical forces in fetal lung development is well established [1,2]. The fetal lung actively secretes fluid into the tissue lumen creating a constant transpulmonary pressure in the potential airway and airspaces of around 2.5 mmHg [3]. Studies in fetal sheep have shown that tracheal ligation, which increases lung distension, caused acceleration of lung development, whereas chronic drainage of tracheal fluid, which inhibits lung distension, retarded lung growth and development [4]. These investigations clearly demonstrate that fetal lung liquid is a major determinant of the development of the lung [3].

In humans, oligohydramnios secondary to prolonged rupture of membranes can cause maternal, fetal, and neonatal complications, including chorioamnionitis, pulmonary hypoplasia, restriction deformities, fetal loss, and complications of extreme prematurity among surviving infants [5]. Experimentally, it has been demonstrated that oligohydramnios compromises fetal lung development in several animal models[6-8]. The likely mechanism is a reduction in the distention of the lung secondary to a decrease of the volume of fluid in the potential airways and air spaces[8,9]. However, the molecular and cellular mechanisms by which a decrease of lung distension compromises lung development are not well understood.

Previous rodent models have addressed the effect of oligohydramnios on lung growth and maturation[10] and expression levels of ROCK2 [11]. However, the full characterization of a mouse model of pulmonary hypoplasia secondary to severe oligohydramnios is missing and this was the objective of these investigations. The main findings of our study are that pulmonary hypoplasiasecondary to oligohydramnios is associated with a decrease in cell size, a change in cell shape, and a compromise in type I cell differentiation. In addition, oligohydramnios affects angiogenesis and apoptosis without altering cell proliferation. These findings suggest that type I epithelial cells could have an unrecognized key role in the development of the distal lung epithelium mediated by mechanical signals.

Materials and Methods

Animal experiments to induce oligohydramnios

All surgical procedures were approved by the Lifespan Institutional Animal Care and Use Committee (IACUC) of Rhode Island. To induce pulmonary hypoplasia by severe oligohydramnios, we followed a previously described model [11]. Briefly, timed-pregnant Swiss Webster (SW) mice at E14.5 of gestation underwent median laparotomy under general anesthesia with isoflurane. Amniotic sacs from one uterine horn were punctured using a sterile $22^{1/2}$-gauge needle. The perforation resulted in a rapid and abundant leakage of amniotic fluid. Fetuses in corresponding positions in the opposite horn served as controls (UnT). Fetuses were delivered by cesarean section 4 days after surgery (at E18.5). Lungs were collected (right superior lobe) and either fixed overnight in formalin 10% and subsequently embedded in paraffin or immediately frozen in liquid nitrogen (rest of the lung) and store at -80^{0}C for further analysis.

Lung morphometry

Digital images of the H&E stained sections of the lung were captured at 40X magnification using a Zeiss-Axio Imager upright microscope and an AxioCam MRc digital camera. The image processing and analysis was conducted using ImageJ (v 1.5g) (NIH, Bethesda, MD). To assess the size of the spaces within the lung tissue, a total of 54 images were analyzed. These included OH (n= 30 images from 10 fetuses) and UnT (n= 24 images from 9 fetuses). A Guassian Blur Filter (Sigma radius =4) was applied to the green channel of the images (**Figure 2B and 2C**). The spaces were then isolated using a multilevel thresholding procedure [12]. The area and perimeter for the spaces were analyzed [13]. To analyze the size and morphological features of the fetal pulmonary cells, spaces (isolated as described above) were subtracted from the 40X images and the remaining tissue was segmented into theoretical cell areas (virtual cells or v-cells) as described [14-16]. Briefly, nuclei were localized based on the optical density of hematoxylin after color deconvolution of the H&E images [17]. Nuclei were then converted into nuclear "seeds" using greyscale reconstruction [18] and a watershed transform [19] was then applied to segment the tissues into areas of influence exclusive to each nuclear seed (**Figure 3A**). Morphologic properties of the v-cells were assessed, mainly the v-cell perimeter, area, Feret's diameter, roundness, circularity and sphericity [13]. Images were analyzed from two independent experiments. The total number of v-cells analyzed was (n=2683 for OH and n=1828 for UnT).

Statistical analysis

Statistical parameters were calculated using GraphPad Prism and Microsoft Excel. Statistical significance was determined by unpaired t tests ($P < 0.05$ was considered statistically significant).

For additional information on *Animal surgery*, *Lung wet weight/dry weight ratio (W/D), DNA quantification, Antibodies* used in these studies, *Immunohistochemistry, Flow Cytometry, Western blot*, *Real Time PCR,* and *TUNEL assay* experiments please see on line supplemental material.

Results

Oligohydramnios compromises the growth of the fetal lung without altering the DNA content.

First, we assessed the effect of OH on the weight of the fetal lungs and how it relates to the overall body weight in comparison to the untouched (UnT) controls. Fetuses and lungs were weighed and lung tissues were extracted at E18.5. We found that the total body weight was significantly reduced in OH (1063mg ± 21.56) mice when compared to UnT controls (1199mg ± 20.60). The lung weight was also significantly reduced in OH (24.95mg ± 0.7353) when compared to UnT (31.78mg ± 0.943), resulting in a significantly lower lung to total body weight ratio (**Figure 1A**). Moreover, lung water was significantly reduced in oligohydramnios fetuses when compared to controls (6.63mg ± 0.43 versus 8.13mg ± 1.12) (**Figure 1B**). In contrast, the total amount of DNA did not differ between oligohydramnios and control fetuses (47mg ± 33.5 versus 47.3mg ± 19.2) (**Figure 1C**). These results demonstrate that OH compromises the growth/development of the fetal lung by decreasing the lung water content without affecting the amount of DNA.

Oligohydramnios reduces the distal air space

To further investigate whether OH induces pulmonary hypoplasia, H&E lung sections from OH and UnT controls were analyzed. Our data showed histological differences between OH and UnT. In particular, the distal air space was clearly affected (**Figure 2A and 2B**). To quantify these differences, digital images were obtained and the space sizes from OH and UnT samples were analyzed. **Figure 2C** shows the binary images of the histological sections shown in **Figure 2B**. These images were used to determine various descriptors of the space size. Two parameters of size, cells area and perimeter, were significantly reduced in OH when compared to UnT controls. The average cell area for OH was 6546 pixels ± 338 compared to 24668 pixels ± 2156 in UnT. The average cell perimeter for OH was 500 pixels ± 13.66 and 787.4 pixels ± 34.47 for UnT (**Figure 2D**).

Oligohydramnios compromises cell size and shape

To further assess histological differences between OH and UnT, cellular morphology parameters were investigated; specifically cell size and shape. H&E images were analyzed by segmenting the tissue area automatically into v-cells based on the nuclear density and using a watershed transform (**Figure 3A**). Our results showed a significant difference in the cell size between OH and UnT, where cells in OH were smaller than UnT. This was determined by cell area (640.5 pixels ± 9.48 *vs.* 818.5 pixels ± 14.18), perimeter (106.7 pixels ± 0.79 *vs*. 120.2 pixels ± 1.09), and Feret diameter (largest axis length) (37.78 pixels ± 0.27 *vs.* 42.09 pixels ± 0.36) (**Figure 3B**). When the shape of the cells was analyzed, cells in OH were marginally, but significantly less regular than those in UnT, as they displayed

significantly lower values of sphercity (0.42 ± 0.002 *vs.* 0.44 ± 0.003), circularity (0.62 ± 0.002 *vs.* 0.63 ± 0.003) and roundness (0.50 ± 0.002 *vs.* 0.52 ± 0.003) (**Figure 3C**).

Oligohydramnios affects the differentiation of Type I but not Type II cells

We next investigated the impact of severe OH on the differentiation of the distal type I and type II epithelial cells. The expression of T1-α, a marker for alveolar type I cells, was assessed at the protein and mRNA levels. Our Western blot data show that T1-α is significantly reduced in fetuses with OH (218.9 ± 11.77) when compared to UnT controls (248.8 ± 2.48) (**Figure 4A**). Furthermore, the percentage of cells expressing T1-α, as analyzed by flow cytometry, was also significantly reduced (34.20% ± 0.0 vs 18.03% ± 4.288) (**Figure 4B**). Immunohistochemistry studies confirmed that the number of type I cells is reduced in OH lung tissue when compared to UnT (**Figure 4C**). The reduction in T1-α was also observed at the mRNA level (1.07 ± 0.04 vs 0.42 ± 0.06) (**Figure 4D**). These findings clearly demonstrate that OH affects the differentiation of Type I cells.

After that, we studied the effect of OH on differentiation of alveolar type II epithelial cells, using SP-C as a marker. Our results demonstrate that OH did not affect SP-C protein levels (216.6 ± 11.95 *vs.* 221.8 ± 8.26) (**Figure 5A**). Furthermore, the percentage of the cells expressing SP-C was not altered by OH when analyzed by flow cytometry (data not shown). This was further confirmed by immunohistochemistry, where no differences in the number of cells expressing SP-C were shown between OH and UnT fetal lung tissue (**Figure 5B**). Similar results were observed at the mRNA level (1.28 ± 0.18 vs 0.99 ± 0.26) (**Figure 5C**). Altogether, these findings show that OH interferes with the differentiation of alveolar type I epithelial cells without affecting alveolar type II cell differentiation.

Oligohydramnios does not affect proliferation but decreases apoptosis

Next, we studied whether induction of pulmonary hypoplasia in our model affects proliferation and apoptosis. We found that the expression of Proliferating Cell Nuclear Antigen (PCNA) was not affected by OH by Western blot (217 ± 4.9 *vs.* 199 ± 9.6) (**Figure 6A**) or by flow cytometry (74.98 ± 11.1 *vs.* 65.27 ± 9.6) (**Figure 6B**). In contrast, when apoptosis was assessed by Caspase 3 abundance, there was a remarkable decrease in the percentage of cells expressing this protease in OH when compared to UnT, indicating a decrease in the overall number of apoptotic cells (**Figure 6C**). This was further confirmed using a TUNEL assay; where there were a significantly lower number of apoptotic cells in OH when compared to UnT (**Figure 6D**).

Oligohydramnios compromises angiogenesis

We have shown that Oligohydramnios causes pulmonary hypoplasia and compromises the development of distal lung. To further investigate whether angiogenesis was affected, the expression level of endomucin, an endothelial marker, was analyzed. Our data show that protein level of endomucin in the lung tissue of OH samples was decreased when compared to Unt controls (217.2 ± 14.5 vs 168.9 ± 11) (**Figure 7A**). Moreover, immunohistochemistry studies in OH showed a decrease of the number of cells expressing endomucin. In fact, OH has fewer subepithelial cells positive for endomucin and fewer intercapillary connections than UnT. Furthermore, double layers in capillaries were only present in UnT samples (**Figure 7B**). All these findings suggest a defect in angiogenesis in OH.

Discussion

Pulmonary hypoplasia secondary to oligohydramnios can cause significant morbidity and mortality to the neonatal population [5]. The management of this condition is primarily supportive with no current treatment available to stimulate the development of the lung. In order to test specific therapies, an important prior step is to develop animal models of pulmonary hypoplasia that mimic human conditions. In the present study, we fully characterized a mouse model of pulmonary hypoplasia induced by oligohydramnios at the histological, cellular, and molecular levels. The main findings of our study are that pulmonary hypoplasia secondary to oligohydramnios decrease cell size, alter cell shape and compromise type I cell differentiation. In addition, oligohydramnios affects angiogenesis and apoptosis without altering cell proliferation. Some of these findings mimic the phenotype of mice lacking T1α,[20,21] suggesting that type I epithelial cells could have an unrecognized key role in the development of the distal lung epithelium mediated by mechanical signals.

The present investigations show that severe oligohydramnios retards the growth of fetal body and lungs, but the effect is greater on the lungs, as shown by the lower lung weight-to-body weight ratio in the fetuses subjected to oligohydramnios. Our results are in agreement with previous studies observing similar findings [7,10]. In addition, the induction of OH significantly reduced lung water content and compromised the size of the distal air spaces. This was demonstrated by a decrease of wet/dry weight ratio and reduction in the area and perimeter of spaces in OH animals when compared to UnT controls. Accordingly, the decrease of lung weight, not explained by any reduction in proliferation, change on DNA content or increased apoptosis, could be interpreted as a decrease of distension of the fetal lung secondary to smaller volume of fluid in the potential airways and air spaces [8]. Therefore, oligohydramnios causes a decrease in lung distension that retards lung development without altering cell proliferation.

Our data also show that v-cells segmented from histological images of fetal lung tissues display significantly smaller sizes in OH when compared to UnT. This was confirmed using multiple parameters that estimate cell size. In addition, we also observed that v-cells in OH are less regular in shape than UnT, as determined by various measurements of cell morphology. These irregularities of cell morphology are considered a sign of cellular response to stress [22]. In this case, the stress could be related to an increase of external compression secondary to smaller volume of fluid in the lung. This factor could comprise the wellbeing of these cells. Reports of changes in the morphology of fetal cells due to prenatal stress have been reported in microglia [23,24]. Here, we report for the first time that changes in the morphology of prenatal pulmonary cells occur as a result of OH. One limitation of these studies is the inability to specify the celltype we are analyzing. In fetal tissues, as many cells are actively dividing, the nuclei have similar intensity and therefore difficult to distinguish from one another unless specific markers are used. However, and for the same reasons, all the cells were captured by the thresholding method based on the nuclear intensity.

Our investigations also identified a significantly lower percentage of fetal lung cells expressed T1-α in the OH lungs when compared to controls, as previously described[10]. T1alpha, a lung type I cell differentiation gene, is developmentally regulated and expressed only in type I cells. Type I cells cover a large surface areain the developed lung [25], and are important in many of the lung functions such as gas exchange [26], fluid balance in the alveolus [27], and innate immunity [28,29]. Studies from T1α knockout mice indicate that alveolar type I cells may be also critical for normal lung development[20,30] since T1α knockout mice died at birth of respiratory failure and histologic analysis show underdeveloped lungs[20].

The typical flat morphology of type I cells begin to appear in the late canalicular period and increase in number during the saccular and alveolar stages of lung development[31]. Previous

investigations believed that type I cells are derived from type II cells[32,33]. However, recent studies using specific markers for type I (T1α) and type II cells (SP-C) have clearly shown the presence in the distal lung of alveolar progenitor cells containing both phenotypes, before they became differentiated type I or type II cells[34]. Therefore, these studies demonstrate that during fetal lung development, alveolar type I and type II epithelial cells are derived from a bipotent progenitor cell[34]. Hooper's group found that the phenotypes of type-I and type-II alveolar epithelial cells are strongly influenced by the basal degree of lung expansion. They observed a rise in the number of type I cells after increase of fluids induced by tracheal occlusion, and the opposite was found when fluids were drained from the lung [35]. Therefore, our data are consistent with these investigations, demonstrating a decrease in the number of type I cells after lung drainage induced by OH. Another important point derived from these studies, and not previously fully recognized, is the potential key role of type I epithelial cells in the differentiation of the distal lung epithelium mediated by mechanical signals. This is supported by studies in acceleration of lung development models, such as tracheal occlusion, where type I phenotype is stimulated. In addition, T1-alpha knockout mice show alveolar underdevelopment. We speculate that fluids distension may be critical to determine the phenotype of bipotent progenitor cells and the lack of distention may alter the ability for bipotent cells to differentiate into type I cells and compromise cell signaling in the distal epithelium, critical for normal lung development. However, this hypothesis needs to be tested experimentally.

In contrast, OH did not affect the number of cells expressing the type II cell marker SP-C, at the protein or at the transcription levels. Our results are consistent with previous studies showing no changes in Type II cell differentiation after OH [10]. Similar results were also found in the T1-aplha knockout mice [20]. Type II epithelial cells are capable of differentiating into type I cells upon lung injury after birth [36,37]. However, this compensatory effect does not

occur prenatally, and the population of type I cells are derived only from bipotent progenitor cells [34,38]. Therefore, it was not a surprise to find that in OH, there was not an increase of type II cells to compensate for type I deficiency.

Our studies showed no differences in proliferation or DNA content between OH and control lungs. Given that OH affects lung growth one can intuitively think that proliferation would be decreased in this model. However, as we discussed before, the effect of OH in lung size is mostly due to decrease of fluids inside the lung. Moreover, in acceleration of lung development by tracheal occlusion there is an increase of cell proliferation[39]; therefore, it is not a surprise that in the opposite model proliferation is not increased.

We found that apoptosis is decreased in OH lung tissue in comparison to UnT. These results are consistent with previous studies from our laboratory demonstrating that mechanical signals increase apoptosis and promote distal lung remodeling by thinning the mesenchyme and bringing epithelium and endothelium into apposition[40]. Therefore, the decrease of mechanical signals by less lung distension could affect apoptosis in the opposite direction. However, this sentence remains speculative given that apoptosis was not investigated in a cell-specific manner.

Our studies demonstrate that angiogenesis is compromised in the OH model of pulmonary hypoplasia. The marked reduction in the size of the distal air spaces is associated with a significant decrease in the expression of endomucin, an endothelial marker. This correlates perfectly with the defect in blood vessel expansion. Furthermore, there was a failure of capillary formation within OH lung tissue when compared to controls. All these factors lead us to conclude that in this surgically induced OH model, a defect in blood vessel growth plays an important role in the development of pulmonary hypoplasia. Given that epithelial-

endothelial crosstalk is critical for distal lung development and that type II epithelial cells are not affected; we speculate that type I epithelial cells could have also an important role in angiogenesis via epithelial-endothelial signaling. In fact, recent studies have demonstrated that type I epithelial cells are a source of VEGFA and their normal development is required for alveolar angiogenesis[41].

In summary, here we have fully characterized a murine model of pulmonary hypoplasia induced by severe oligohydramnios. We found that OH decreases cell size, alters cell shape, and compromises type I cell differentiation. In addition, oligohydramnios affects angiogenesis and apoptosis without altering cell proliferation. Therefore, this model would be important to investigate the role of type I epithelial cells in distal lung development and potential rescue therapies for pulmonary hypoplasia.

References

1. Kitterman JA. The effects of mechanical forces on fetal lung growth. Clin Perinatol 1996;23(4):727 PubMed -740.
2. Moessinger AC, Harding R, Adamson TM, Singh M, Kiu GT. Role of lung fluid volume in growth and maturation of the fetal sheep lung. J Clin Invest 1990;86(4):1270 PubMed -1277.
3. Hooper SB, Harding R. Fetal lung liquid: a major determinant of the growth and functional development of the fetal lung. Clinical and experimental pharmacology & physiology 1995;22(4):235 PubMed -247.
4. Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM. Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. J Anat 1977;123(3):649 PubMed -660.
5. Fliegner JR, Fortune DW, Eggers TR. Premature rupture of the membranes, oligohydramnios and pulmonary hypoplasia. Aust N Z J Obstet Gynaecol 1981;21(2):77-81.
6. Moessinger AC, Collins MH, Blanc WA, Rey HR, James LS. Oligohydramnios-induced lung hypoplasia: the influence of timing and duration in gestation. Pediatr Res 1986;20(10):951 PubMed -954.
7. Moessinger AC, Bassi GA, Ballantyne G, Collins MH, James LS, Blanc WA. Experimental production of pulmonary hypoplasia following amniocentesis and oligohydramnios. Early Hum Dev 1983;8(3-4 PubMed):343-350.
8. Dickson KA, Harding R. Decline in lung liquid volume and secretion rate during oligohydramnios in fetal sheep. J Appl Physiol (1985) 1989;67(6 PubMed):2401-2407.
9. Savich RD, Guerra FA, Lee CC, Padbury JF, Kitterman JA. Effects of acute oligohydramnios on respiratory system of fetal sheep. J Appl Physiol (1985) 1992;73(2 PubMed):610-617.
10. Kitterman JA, Chapin CJ, Vanderbilt JN, Porta NF, Scavo LM, Dobbs LG, Ertsey R, Goerke J. Effects of oligohydramnios on lung growth and maturation in the fetal rat. Am J Physiol Lung Cell Mol Physiol 2002;282(3):L431-439.
11. Cloutier M, Tremblay M, Piedboeuf B. ROCK2 is involved in accelerated fetal lung development induced by in vivo lung distension. Pediatric pulmonology 2010;45(10):966-976.
12. Otsu N. A threshold selection method from grey-level histograms. IEEE Trans Syst Man Cybern 1979;9(1):62-66.
13. Landini G. Advanced shape analysis with imageJ. The Second ImageJ User and Developer Conference. Luxembourg2008. p 116-131.
14. Abu Eid R, Sawair F, Landini G, Saku T. Age and the architecture of oral mucosa. Age (Dordr) 2012;34(3):651 PubMed -658.
15. Abu Eid R, Landini G. Morphometry of pseudoepitheliomatous hyperplasia: objective comparison to normal and dysplastic oral mucosae. Anal Quant Cytol Histol 2005;27(4):232-240.
16. Abu-Eid R,Landini G. Morphometrical differences between pseudo-epitheliomatous hyperplasia in granular cell tumours and squamous cell carcinomas. Histopathology 2006;48(4):407 PubMed -416.

17. Ruifrok AC, Johnston DA. Quantification of histochemical staining by color deconvolution. Anal Quant Cytol Histol 2001;23(4):291-299.
18. Landini G, Othman IE. Estimation of tissue layer level by sequential morphological reconstruction. J Microsc 2003;209(Pt 2):118-125.
19. Vincent L, Soille P. Watersheds in digital spaces: An efficient algorithm based on immersion simulations. IEEE Trans Pattern Anal Mach Intell 1991;13:583-598.
20. Ramirez MI, Millien G, Hinds A, Cao Y, Seldin DC, Williams MC. T1alpha, a lung type I cell differentiation gene, is required for normal lung cell proliferation and alveolus formation at birth. Dev Biol 2003;256(1):61 PubMed -72.
21. Millien G, Spira A, Hinds A, Wang J, Williams MC, Ramirez MI. Alterations in gene expression in T1 alpha null lung: a model of deficient alveolar sac development. BMC Dev Biol 2006;6:35.
22. Fulda S, Gorman AM, Hori O, Samali A. Cellular stress responses: cell survival and cell death. Int J Cell Biol 2010;2010:214074.
23. Diz-Chaves Y, Pernia O, Carrero P, Garcia-Segura LM. Prenatal stress causes alterations in the morphology of microglia and the inflammatory response of the hippocampus of adult female mice. J Neuroinflammation 2012;9:71.
24. Slusarczyk J, Trojan E, Glombik K, Budziszewska B, Kubera M, Lason W, Popiolek-Barczyk K, Mika J, Wedzony K, Basta-Kaim A. Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. Front Cell Neurosci 2015;9:82.
25. Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. Am J Respir Cell Mol Biol 1992;6(2):235-243.
26. Makanya A, Anagnostopoulou A, Djonov V. Development and remodeling of the vertebrate blood-gas barrier. BioMed research international 2013;2013:101597.
27. Johnson MD, Bao HF, Helms MN, Chen XJ, Tigue Z, Jain L, Dobbs LG, Eaton DC. Functional ion channels in pulmonary alveolar type I cells support a role for type I cells in lung ion transport. Proc Natl Acad Sci U S A 2006;103(13):4964-4969.
28. Wong MH, Chapin OC, Johnson MD. LPS-stimulated cytokine production in type I cells is modulated by the renin-angiotensin system. Am J Respir Cell Mol Biol 2012;46(5):641-650.
29. Wong MH, Johnson MD. Differential response of primary alveolar type I and type II cells to LPS stimulation. PLoS One 2013;8(1): PubMed e55545.
30. Najrana T, Sanchez-Esteban J. Alveolar Type I Epithelial Cells: The forgotten cells in fetal lung development and lung injury. Pulm Res Respir Med Open J 2016;2(4):e6-e9.
31. Flecknoe SJ, Wallace MJ, Cock ML, Harding R, Hooper SB. Changes in alveolar epithelial cell proportions during fetal and postnatal development in sheep. Am J Physiol Lung Cell Mol Physiol 2003;285(3):L664-670.
32. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Renewal of alveolar epithelium in the rat following exposure to NO2. Am J Pathol 1973;70(2):175 PubMed -198.
33. Gabazza EC, Kasper M, Ohta K, Keane M, D'Alessandro-Gabazza C, Fujimoto H, Nishii Y, Nakahara H, Takagi T, Menon AG, Adachi Y, Suzuki K, Taguchi O. Decreased expression of aquaporin-5 in bleomycin-induced lung fibrosis in the mouse.Pathol Int 2004;54(10):774-780.

34. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 2014;507(7491):190 PubMed -194.
35. Flecknoe SJ, Wallace MJ, Harding R, Hooper SB. Determination of alveolar epithelial cell phenotypes in fetal sheep: evidence for the involvement of basal lung expansion. J Physiol 2002;542(Pt 1):245-253.
36. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, Stripp BR, Randell SH, Noble PW, Hogan BL. Type 2 alveolar cells are stem cells in adult lung. J Clin Invest 2013;123(7):3025 PubMed -3036.
37. Yee M, Gelein R, Mariani TJ, Lawrence BP, O'Reilly MA. The Oxygen Environment at Birth Specifies the Population of Alveolar Epithelial Stem Cells in the Adult Lung. Stem Cells 2016;34(5):1396 PubMed -1406.
38. Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, Desai TJ, Krasnow MA, Quake SR. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature 2014;509(7500):371 PubMed -375.
39. Maltais F, Seaborn T, Guay S, Piedboeuf B. In vivo tracheal occlusion in fetal mice induces rapid lung development without affecting surfactant protein C expression. Am J Physiol Lung Cell Mol Physiol 2003;284(4):L622-632.
40. Sanchez-Esteban J, Wang Y, Cicchiello LA, Rubin LP. Cyclic mechanical stretch inhibits cell proliferation and induces apoptosis in fetal rat lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 2002;282(3):L448-456.

41. Yang J, Hernandez BJ, Martinez Alanis D, Narvaez del Pilar O, Vila-Ellis L, Akiyama H, Evans SE, Ostrin EJ, Chen J. The development and plasticity of alveolar type 1 cells. Development 2016;143(1):54 PubMed -65.

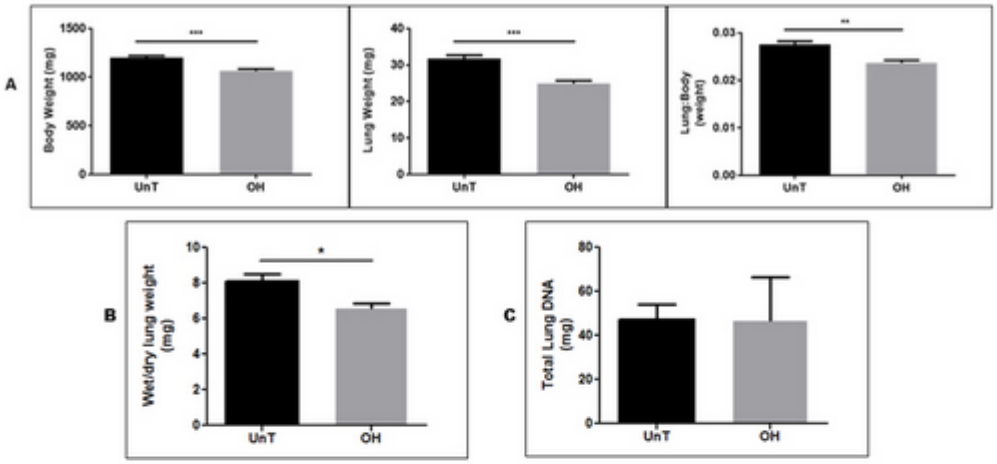

Figure 1. Effect of oligohydramnios on body weight, lung weight and DNA content

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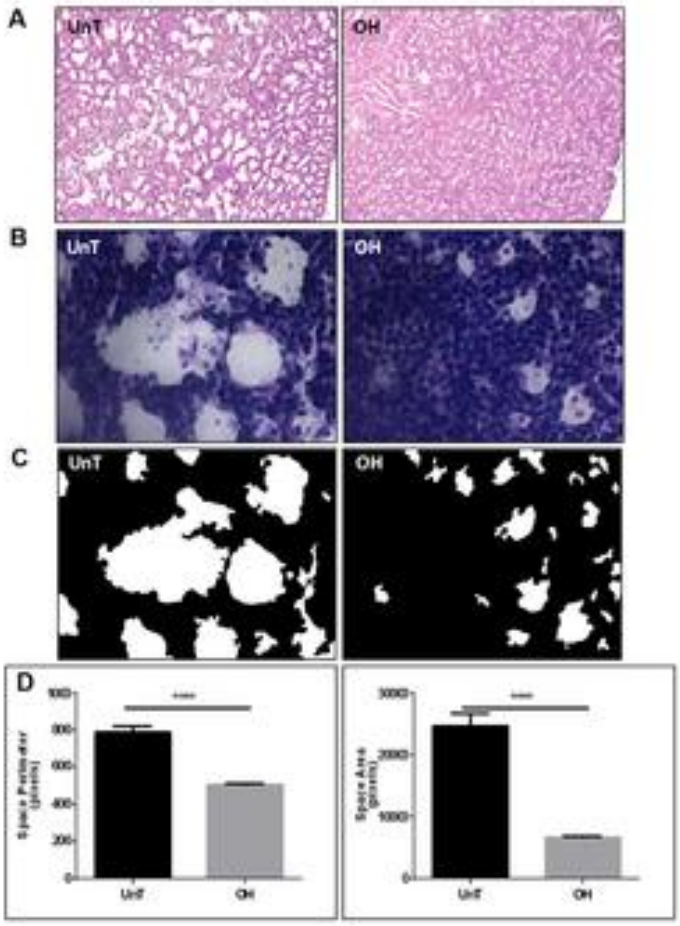

Figure 2. Effect of oligohydramnios on distal air space

87x118mm (72 x 72 DPI)

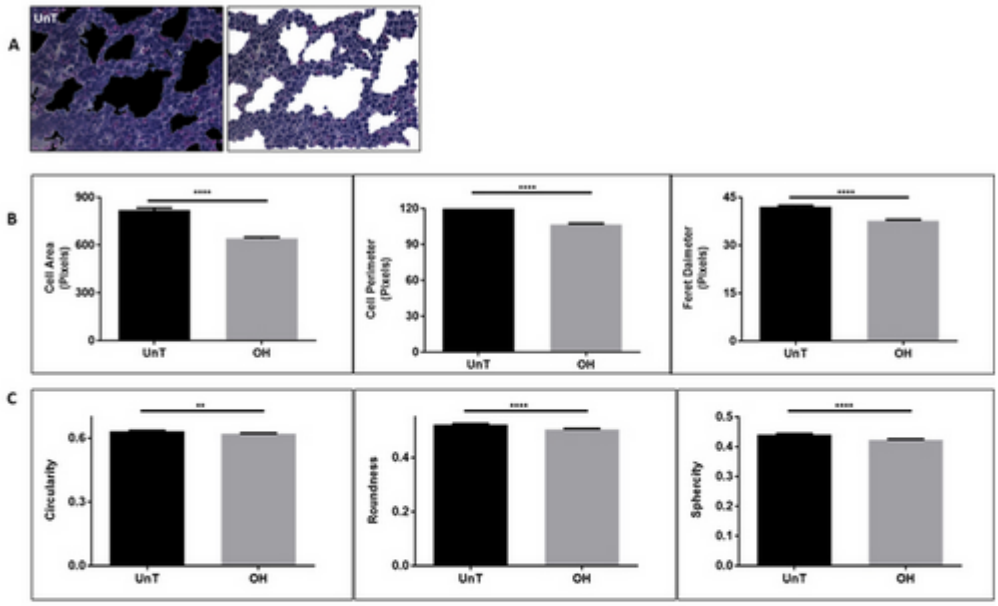

Figure 3. Oligohydramnios affects pulmonary cell size and shape

254x190mm (72 x 72 DPI)

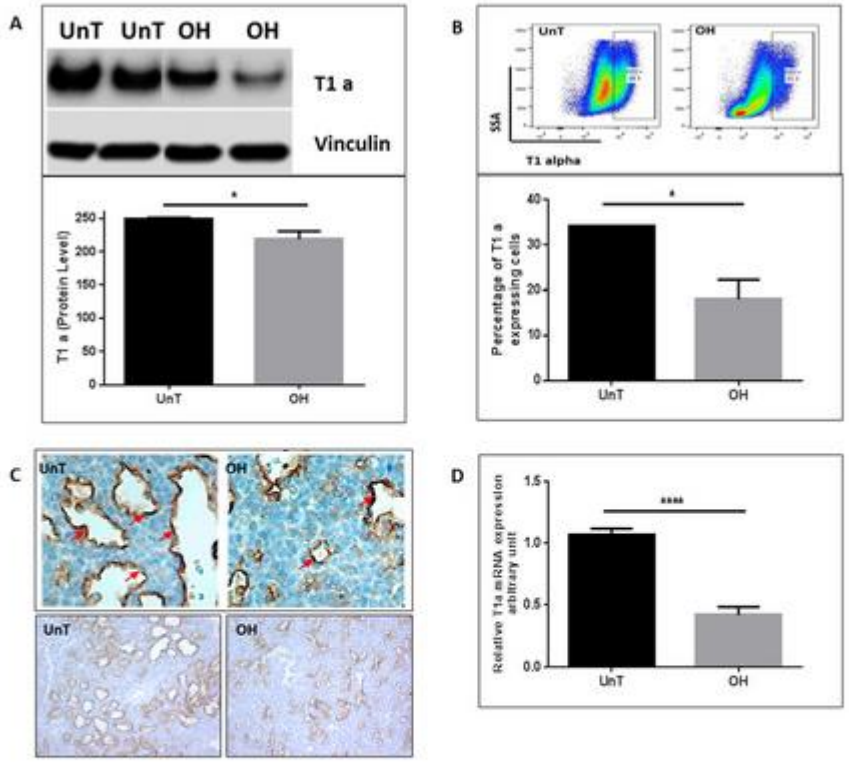

Figure 4. Oligohydramnios significantly reduces alveolar type I cell differentiation

304x254mm (72 x 72 DPI)

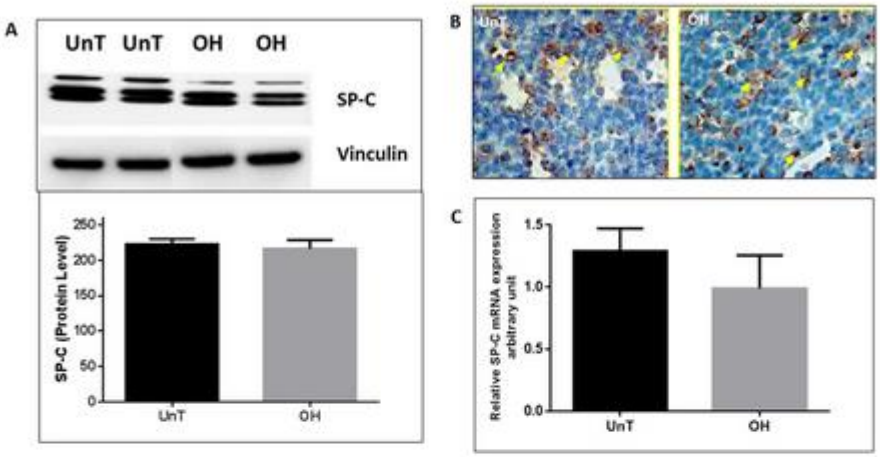

Figure 5. Oligohydramnios does not affect type II cell differentiation

304x254mm (72 x 72 DPI)

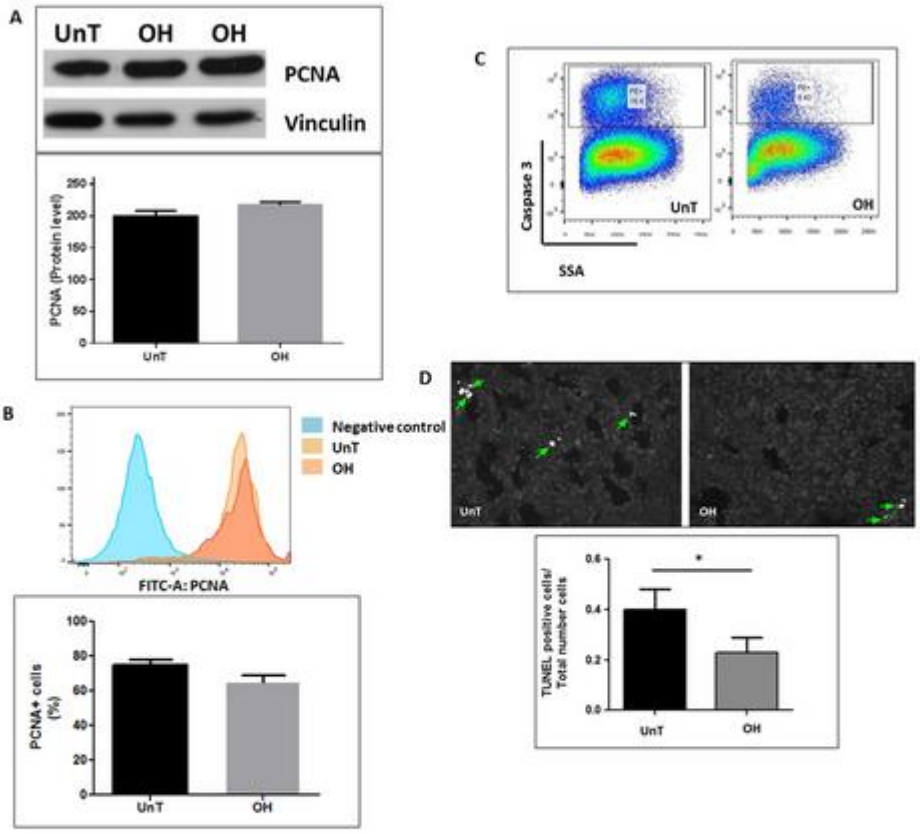

Figure 6. Oligohydramnios does not affect proliferation but reduces apoptosis

304x254mm (72 x 72 DPI)

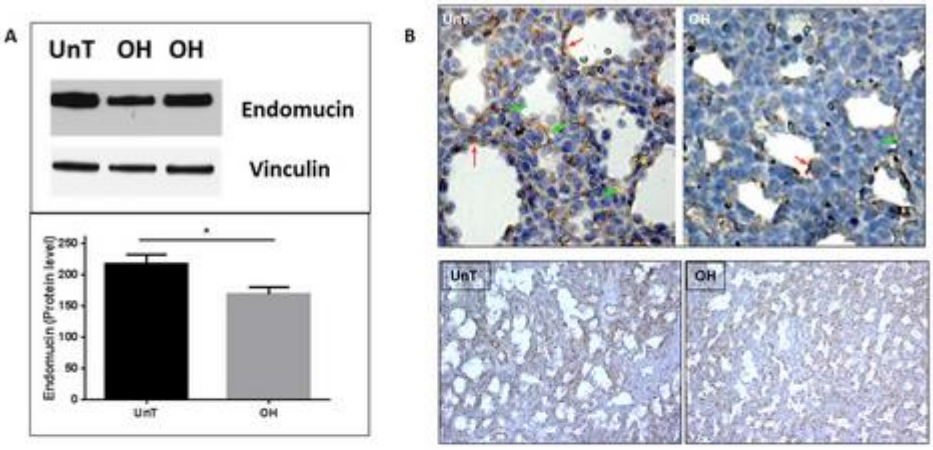

Figure 7. Oligohydramnios affects lung angiogenesis

304x254mm (72 x 72 DPI)