Pulmonary Artery Pressure, Gender, Menopause, and Pregnancy in Schistosomiasis-Associated Pulmonary Hypertension

Anderson C. Armstrong¹,²,³, Ângela M. P. Bandeira², Luis C. L. Correia⁴, Humberto C. O. Melo², Carlos A. M. Silveira², Eugênio Albuquerque², Jeová C. Moraes Jr.³, Antônio M. L. Silva³, João A. C. Lima¹, Dário C. Sobral Filho²

Escola de Medicina Johns Hopkins - Divisão de Cardiologia¹, Baltimore, MD; Universidade de Pernambuco – Faculdade de Ciências Médicas², Recife, PE; Universidade Federal do Vale do São Francisco - Colegiado de Medicina¹, Petrolina, PE; Escola Bahiana de Medicina e Saúde Pública⁴, Salvador, BA – Brazil

Abstract

Background: Schistosomiasis-associated pulmonary arterial hypertension (SPAH) is a major concern worldwide. However, the role of gender-specific contributing factors in SPAH is unknown.

Objective: We investigated how systolic pulmonary artery pressure (SPAP) values and the presence of severe SPAP relate to gender, menopausal status, and pregnancy history in SPAH patients.

Methods: Seventy-nine patients diagnosed with SPAH from 2000 to 2009 were assessed and 66 were enrolled in the study. Information about age, menopausal status, pregnancy, echocardiography-derived SPAP, and invasive mean pulmonary artery pressure (mPAP) was collected from medical records. The relation between values of SPAP and mPAP and their agreement for severe disease were assessed. Regression models assessed the association of gender, menopausal status, and pregnancy history with SPAP values and the presence of severe SPAP.

Results: Moderate correlation and good agreement for severe disease were found between mPAP and SPAP. Mean SPAP values were similar for men and women. A trend toward higher values of SPAP was found for non-menopausal women compared to men. Higher SPAP values were found for menopausal compared to non-menopausal women; the values were non-significant after adjustment for age. Pregnancy history had no association with SPAP. Menopause and positive pregnancy had no association with severe SPAP.

Conclusion: In SPAH patients, neither gender, nor menopausal status, nor pregnancy history showed independent correlation with SPAP values assessed by echocardiography. (Arq Bras Cardiol. 2013;101(2):154-159)

Keywords: Hypertension, Pulmonary; Schistosomiasis; Pregnancy; Menopause; Echocardiography, Doppler.

Acronym and abbreviation list

mPAP - mean pulmonary artery pressure
PAH - pulmonary arterial hypertension
SPAP - pulmonary artery systolic pressure
SPAH - schistosomiasis-associated pulmonary arterial hypertension

Introduction

Schistosomiasis affects about 207 million people worldwide and is known as a potential trigger for the pulmonary arterial hypertension (PAH) immune-inflammatory pathway.

Schistosomiasis-associated PAH (SPAH) is recognized as one of the most important etiologies of PAH - with a prevalence of approximately 5% in patients with hepatosplenic schistosomiasis. PAH is a high-risk condition, related to elevated rates of mortality¹-⁶. However, literature regarding the clinical presentation of SPAH is limited.

Echocardiography-derived systolic pulmonary artery pressure (SPAP) is usually used to screen patients suspected of PAH. Adding right atrial pressure to the pressure gradient between right atrium and ventricle has been validated as a method to assess SPAP over the decades⁷-⁸. In fact, SPAP assessed by Doppler-echocardiography is a well-established method in clinical practice, being widely available, inexpensive, and safe⁹-¹⁵.

In PAH clinical presentation, the role of gender-specific characteristics is controversial. Despite the evidence that female hormonal balance could be related to pulmonary artery pressure, studies have failed to demonstrate significant differences in the prognosis of PAH in men compared to women¹⁶-¹⁸. Moreover, the interest in menopausal aspects of PAH is growing, as postmenopausal women have shown significant susceptibility to elevated pulmonary pressure¹⁹,²⁰.
We investigated how SPAP values and severity relate to gender in patients with SPAH. We also explored the association of SPAP with menopausal status and pregnancy history in the female population. Finally, we compared echocardiography-derived SPAP values to values of invasive mean pulmonary artery pressure (mPAP).

**Methods**

**Study design and population**

We enrolled 89 patients (67% women) who had been diagnosed with SPAH by the Pulmonary Hypertension Group at University of Pernambuco (Recife, Brazil) between January 2000 and November 2009. Investigation for PAH follows the protocol suggested by Gaine and Rubin, including diagnostic right-chamber catheterization to ensure values of mPAP > 25 mmHg and pulmonary capillary wedge pressure (PCWP) < 15 mmHg. The diagnosis of schistosomiasis is individually assessed and based on exposure history and stool samples, with confirmatory rectal biopsy and/or serology in specific cases. In the absence of other etiology, SPAH was defined when schistosomiasis and portal fibrosis were present in a patient with PAH assessed by invasive right-chamber catheterization. Our study was approved by the local ethics committee.

**SPAP assessment**

SPAP data were collected from the first registered echocardiogram performed and interpreted by staff cardiologists at the institution of enrolment. Suboptimal echocardiogram views or inadequate tricuspid regurgitation were present in 4 men and 9 women. The institution’s protocol to assess SPAP dictates the following: (a) patient in the left lateral decubitus position; (b) color flow Doppler imaging is used to identify and guide alignment of the cursor with the tricuspid regurgitant jet; (c) using continuous-wave Doppler, tricuspid regurgitation velocity is assessed from different views to measure peak velocity (v); (d) transtricuspid pressure gradient is calculated using the modified Bernoulli equation (4v²); (e) right atrial pressure is evaluated by assessing the respiratory variation in the diameter of the inferior vena cava (values estimated as 5, 10, or 15 mmHg); and (f) SPAP is calculated by adding the transtricuspid pressure gradient to the atrial pressure estimate. The measurement is not performed if there is evidence of obstruction to pulmonary artery flow, such as pulmonary stenosis or right ventricular outflow obstruction.

**Additional data acquisition**

Information on age, menopausal status, and pregnancy history at the diagnosis of SPAH was collected from medical records on all patients followed in the institution. Information on menopausal status was present in 35 women (58% menopause positive) and data on pregnancy history was collected in 20 women (13 had positive history for pregnancy; 56% vs. 73% for non-menopausal vs. menopausal women, respectively). Invasive mean pulmonary artery pressure (mPAP), assessed by diagnostic right-chamber catheterization, was also collected if the interval to the assessment of echocardiography-derived SPAP did not exceed 6 months (n = 42; 81% females; age 44.5 ± 14.3 years; SPAP 96.7 ± 28.8 mmHg; and mPAP 60.3 ± 14.7 mmHg).

**Statistical analysis**

The software STATA, version 11.2, was used to perform the statistical analysis on a cross-sectional basis. Student’s t-test was used to assess differences between mean values and the z-test was used to assess differences between proportions. Severe SPAP and mPAP were defined as the 4th quartile in both cases (≥ 120 mmHg and ≥ 76 mmHg for SPAP and mPAP, respectively). The relationship between echocardiography-derived SPAP and invasive mPAP was assessed by Pearson’s correlation. Percent agreement and Cohen’s kappa coefficient for severe classification of SPAP and mPAP were also computed.

The relation of SPAP, gender, and menopausal status was assessed using linear regression. Logistic regression was used to compute odds ratios for severe SPAP, according to gender and menopausal status. Regression models were performed on univariate and multivariate bases, adjusted to age at diagnosis. Menopausal status as a predictor of SPAP values was compared to male gender and as a binary variable in a female-only subset. For a subgroup of women diagnosed with SPAH, an additional analysis assessed the influence of pregnancy history (used as a binary variable) in the regression models.

**Results**

A total of 66 patients with data on SPAP were included. Patient characteristics are shown in Table 1, arranged according to gender and menopausal status. A moderate correlation was found between mPAP and SPAP (r = 0.51, p < 0.001; Figure 1). In this group, both methods classified 10 cases as severe SPAH, agreeing in 82% of the classifications (Kappa coefficient = 0.3; p < 0.01). Mean SPAP values were similar for men and women (Table 1). In addition, no significant difference was found when menopausal women were compared to men. However, higher values of SPAP were found for non-menopausal women compared to men, with a trend in significance (Table 2).

Among females, statistically significant higher mean SPAP values and ages were found for menopausal women (Table 1). However, the differences become non-significant after adjustment for age. The mean SPAP was 118.3 ± 32.3 mmHg and 95.1 ± 26.2 mmHg (p = 0.1) for women without and with previous pregnancy, respectively. No statistical significance was found when history of pregnancies was added as a covariate (Table 2).

Considering the prediction of severe SPAH (SPAP > 120 mmHg), the presence of menopause and a positive history for pregnancy were consistently associated with low odds ratios; however, although trends were present, no statistically significant odds ratio was reported (Table 3).
Table 1 – Characteristics of patients with schistosomiasis-associated pulmonary hypertension, arranged according to gender and menopausal status

| Variable              | Gender (mean ± SD) | Menopausal status (mean ± SD) |
|-----------------------|--------------------|-------------------------------|
|                       | Men (n = 15)       | Women (n = 51)                | Absent (n = 15) | Present (n = 20) | p       |
| Age at diagnosis (years) | 46.8 ± 12.8       | 45.9 ± 15.1                | 29.4 ± 6.8     | 58.4 ± 9.8       | < 0.0001 |
| SPAP (mmHg)           | 87.8 ± 31.6        | 93.8 ± 28.3              | 110.7 ± 26.0   | 86.5 ± 20.0      | 0.003   |
| Number of pregnancies | NA                 | 3.2 ± 2.8                 | NA             | 1.6 ± 0.5        | 4.2 ± 3.2 | 0.098   |

SD: standard deviation; SPAP: systolic pulmonary artery pressure; NA: non-applicable. Mean number of pregnancies excludes patients without history of pregnancy (7 cases overall; 2 with positive menopausal status). T-test was used to acquire p-values.

Discussion

More than 770 million people worldwide are at risk of contracting schistosomiasis. The prevalence of elevated pulmonary pressures in patients with schistosomiasis is still controversial, but it has been reported as one of the most important etiologies of PAH. This is the first study to assess the influence of gender on echocardiography-derived SPAP and to investigate the role of menopausal status and pregnancy history among patients diagnosed with SPAH.

The estimation of SPAP by Doppler echocardiography is validated, safe, usually feasible, and universally adopted in screening protocols for PAH. We showed a moderate correlation between echocardiography-derived SPAP and invasive measurement of mPAP in patients with SPAH (Figure 1), with 82% of agreement and a fair Kappa coefficient when classifying the most severe cases. mPAP values assessed by right-chamber catheterization are considered the gold standard for the diagnosis of PAH. Similar to our study, diverse reports have shown that the correlation between SPAP values estimated by echocardiography and determined invasively is moderate to strong.
Table 2 – Linear regression models for SPAP (mmHg) according to gender, menopausal status, and pregnancy history

| Predictor                      | n  | Regression coefficients (p value) |
|--------------------------------|----|----------------------------------|
|                                |    |                                  |
|                                |    | Univariate                      | MODEL 1 | MODEL 2 |
| Female (vs. male)              | 66 | 6.0 (0.48)                       | 13.7 (0.09) | NA |
| non-menopausal women (vs. male)| 51 | 22.9 (0.02)                      | 23.4 (0.05) |       |
| Menopausal women (vs. male)    | 51 | -1.3 (0.88)                      | 5.4 (0.59) |       |
| Menopausal present (vs. absent)§| 36 | -24.2 (0.003)                    | -8.4 (0.58) | -38.7 (0.11) |
| Pregnancy present (vs. absent) | 20 | -23.1 (0.10)                     | -13.9 (0.31) | -20.1 (0.15) |

SPAP: systolic pulmonary artery pressure; NA: non-applicable. MODEL 1 – adjusted for age; MODEL 2 – age, menopausal status, and pregnancy history in the same model. § In MODEL 2, analysis used n = 20.

Table 3 – Logistic regression models for severe SPAP (≥ 120 mmHg) according to gender, menopausal status, and pregnancy history

| Predictor                      | n  | Odds Ratio (p value) |
|--------------------------------|----|----------------------|
|                                |    | Univariate           | MODEL 1 | MODEL 2 |
| Female (vs. male)              | 66 | 1.2 (0.77)           | 1.7 (0.51) | NA |
| non-menopausal women (vs. male)| 51 | 2.0 (0.41)           | 5.5 (0.19) |       |
| Menopausal women (vs. male)    | 51 | 0.4 (0.38)           | 0.4 (0.39) |       |
| Menopausal present (vs. absent)§| 36 | 0.2 (0.09)           | 0.4 (0.62) | 0.03 (0.14) |
| Pregnancy present (vs. absent) | 20 | 0.2 (0.14)           | 0.3 (0.27) | 0.1 (0.13) |

SPAP: systolic pulmonary arterial pressure; NA: non-applicable. MODEL 1 – adjusted for age; MODEL 2 – age, menopausal status, and pregnancy history in the same model. § In MODEL 2, analysis used n = 20.

We found a predominance of females in our SPAH patients (67%). A similar predominance of females has been reported for other PAH etiologies, but the real influence of gender in PAH is still controversial. The higher prevalence of PAH in women compared to men, the thrombogenic effects of estrogen, and the fluctuation of estrogen metabolites may indicate potential negative effects of female physiology on the pulmonary vasculature. Estrogen, however, also showed benefits for pulmonary vasculature in both acute and chronic experimental models of pulmonary hypertension. We found similar values of SPAP in men and women with SPAH. Moreover, gender had no statistically significant predictive ability for SPAP when adjusted for age at diagnosis (Table 2).

Pulmonary artery vasoreactivity is affected by changes in estrogen levels, even in a physiological range. Menopausal status represents an important decrease in the circulating estrogen in women. Among patients with systemic sclerosis, postmenopausal women have shown an increased risk for the development of PAH. In fact, the majority of pulmonary hypertensive women in the University of Colorado Pulmonary Hypertension Center were postmenopausal. Our study has intrinsic limitations related to the information on menopausal status collected from medical records. In addition, the real influence of hormonal imbalance could not be assessed, due to the absence of serum hormonal values in our data.

In the subset of SPAH patients who were women, the association between menopausal status and SPAP had no statistical significance when adjusted for age (Table 2). In univariate analysis with SPAH patients, non-menopausal females showed significantly higher SPAP values compared to men; however, this association had borderline statistical significance (p = 0.05) after adjustment for age (Table 2). Our results suggest that the differences found comparing post-menopausal and non-menopausal subjects are due to the influence of age on SPAP. In fact, the positive correlation between SPAP values and age has been seen in a large population of participants free of PAH. In this population, the relationship seems to be mediated by left ventricular diastolic dysfunction and systemic vascular stiffening.

Hormonal and hemodynamic changes that occur during pregnancy contribute to the high maternal mortality in women with pulmonary vascular disease. The influence of previous pregnancies on the clinical presentation of SPAH, however, is unknown. In 20 women at the time of diagnosis for SPAH, we assessed the influence of a positive pregnancy history on the values of SPAP. Thus, the reduced number of patients probably limited the analysis. In our population, positive pregnancy history did not show statistically significant association with SPAP in women with SPAH.
There is evidence that female hormonal imbalance may relate to the severity of pulmonary pressure values. Experimental models showed more severe pulmonary hypertension in chronically hypoxemic rodents that have had their ovaries removed than those with intact ovaries. In this study, the return of estrogens after an ovariectomy led to a regression to baseline levels of pulmonary pressure. Moreover, a history of previous pregnancies showed prognostic value in pregnant patients with pulmonary hypertension. We investigated how gender, menopausal status, and pregnancy history predicted severe SPAP values (> 120 mmHg) in SPAH patients, but found no statistically significant association (Table 3).

Our study contributes to the knowledge of SPAH clinical presentation and its relation to gender, menopausal status, and pregnancy history; however, the role of gender-specific characteristics in the pathological processes of SPAH is yet to be defined. Further studies are needed to establish the relations between gender particularities and SPAH clinical presentation.

Conclusion
In a population of SPAH patients, neither gender, nor menopausal status, nor pregnancy history showed a statistically significant, independent relationship to SPAP values assessed by echocardiography. Differences in SPAP that appear to be related to menopausal status seem to be mediated by age differences.

Author contributions
Conception and design of the research: Armstrong AC, Bandeira AMP, Correia LCL, Lima JAC, Sobral Filho DC; Acquisition of data: Armstrong AC, Bandeira AMP, Melo HCO, Silveira CAM, Albuquerque E, Moraes Jr. JC, Silva AML, Sobral Filho DC; Analysis and interpretation of the data: Armstrong AC, Bandeira AMP, Correia LCL, Melo HCO, Silveira CAM, Albuquerque E, Moraes Jr. JC, Silva AML, Lima JAC, Sobral Filho DC; Statistical analysis: Armstrong AC, Bandeira AMP, Correia LCL, Sobral Filho DC; Writing of the manuscript: Armstrong AC, Lima JAC, Sobral Filho DC; Critical revision of the manuscript for intellectual content: Armstrong AC, Bandeira AMP, Correia LC, Melo HCO, Silveira CAM, Albuquerque E, Moraes Jr. JC, Silva AML, Lima JAC, Sobral Filho DC.

Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Sources of Funding
There were no external funding sources for this study.

Study Association
This article is part of the thesis of master submitted by Anderson da Costa Armstrong, from Universidade de Pernambuco.

References
1. Ross AG, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, et al. Schistosomiasis. N Engl J Med.2002;346(16):1212-20.
2. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. Lancet Infect Dis.2006;6(7):411-25.
3. Ferreira RC, Domingues AL, Bandeira AP, Markman Filho B, Albuqerque Filho ES, Correia de Araujo AC, et al. Prevalence of pulmonary hypertension in patients with schistosomal liver fibrosis. Ann Trop Med Parasitol.2009;103(2):129-43.
4. Souza R, Fernandes CJ, Jardim CV. Other causes of pah (schistosomiasis, porto-pulmonary hypertension and hemolysis-associated pulmonary hypertension). Semin Respir Crit Care Med.2009;30(4):448-57.
5. Lapa M, Dias B, Jardim C, Fernandes CJ, Durado PM, Figueiredo M, et al. Cardiopulmonary manifestations of hepatosplenic schistosomiasis. Circulation.2009;119(11):1518-23.
6. Fernandes CJ, Jardim CV, Hovnanian A, Hoette S, Morinaga LA, Souza R. Schistosomiasis and pulmonary hypertension. Expert Rev Respir Med.2011;5(3):675-81.
7. Yock PG, Popp RL. Noninvasive estimation of right ventricular systolic pressure by doppler ultrasound in patients with tricuspid regurgitation. Circulation.1984;70(4):657-62.
8. Ristow B, Schiller NB. Stepping away from ritual right heart catheterization to the era of noninvasively measured pulmonary artery pressure. J Am Soc Echocardiogr.2009;22(7):820-2.
9. Calliou MR, Ramos PR. Hipertensão arterial pulmonar. Arq Bras Cardiol.2009;93(5 Suppl 1):156-9.
10. Badano LP, Ingelino C, Easaw J, Muraru D, Grillo MT, Lancellotti P, et al. Rightventricle in pulmonary arterial hypertension: haemodynamics, structural changes, imaging, and proposal of a study protocol aimed to assess remodelling and treatment effects. Eur J Echocardiogr.2010;11(1):27-37.
11. Testani JM, St John Sutton MG, Wiegers SE, Kheteral P, Kirkpatrick NJ. Accuracy of noninvasively determined pulmonary artery systolic pressure. Am J Cardiol.2010;105(8):1192-7.
12. Shapiro SM, Oudiz RJ, Cao T, Romano MA, Beckmann XJ, Georgiou D, et al. Primary pulmonary hypertension: improved long-term effects and survival with continuous intravenous epoprostenol infusion. J Am Coll Cardiol.1997;30(2):343-9.
13. Fisher MR, Criner GJ, Fishman AP, Hassoun PM, Minai OA, Scharf SM, et al. Estimating pulmonary artery pressures by echocardiography in patients with emphysema. Eur Respir J.2007;30(5):914-21.
14. Chemla D, de Zuttere D, Herve P. Echocardiographic evaluation of systolic and mean pulmonary artery pressure in patients with pulmonary hypertension. Eur J Echocardiogr.2011;12(12):969.
15. Pyxaras SA, Pinamonti B, Barhadi G, Santangelo S, Valentinic M, Cottolo F, et al. Echocardiographic evaluation of systolic and mean pulmonary artery pressure in the follow-up of patients with pulmonary hypertension. Eur J Echocardiogr.2011;12(9):696-701.
16. D’Alonzo GE, Barst RJ, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Survival in patients with primary pulmonary hypertension: results from a national prospective registry. Ann Intern Med.1991;115(5):343-9.
17. Rich S, Dantzker DR, Ayres SM, Bergofsky EH, Brundage BH, Detre KM, et al. Primary pulmonary hypertension: A national prospective study. Ann Intern Med. 1987;107(2):216-23.

18. Humbert M, Sitbon O, Chaouat A, Bertocchi M, Habib G, Gressin V, et al. Pulmonary arterial hypertension in France: Results from a national registry. Am J Respir Crit Care Med. 2006;173(9):1023-30.

19. Scorza R, Caronni M, Bazzi S, Nador F, Beretta L, Antonioli R, et al. Postmenopause is the main risk factor for developing isolated pulmonary hypertension in systemic sclerosis. Ann NY Acad Sci. 2002;966:238-46.

20. Taraseviciute A, Voelkel NF. Severe pulmonary hypertension in postmenopausal obese women. Eur J Med Res. 2006;11(5):198-202.

21. Gaine SP, Rubin LJ. Primary pulmonary hypertension. Lancet. 1998;352(9129):719-25.

22. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. Vigilância em saúde: dengue, esquistossomose, hanseníase, malária, tracoma, tuberculose. Brasília; 2007. (Cadernos de Atenção Básica nº 21. Série A. Normas e Manuais Técnicos).

23. Graham BB, Bandeira AP, Morrell NW, Butrous G, Tuder RM. Schistosomiasis-associated pulmonary hypertension: pulmonary vascular disease: the global perspective. Chest. 2010;137(6 Suppl):20S-9S.

24. Lopes AA, Bandeira AP, Flores PC, Santana MV. Pulmonary hypertension in Latin America: pulmonary vascular disease: the global perspective. Chest. 2010;137(6 Suppl):78S-84S.

25. Machado C, Britoi D, Souza D, Correia LC. Etiological frequency of pulmonary hypertension in a reference outpatient clinic in Bahia, Brazil. Arq Bras Cardiol. 2009;93(6):629-36, 679-86.

26. Currie PJ, Seward JB, Chan KL, Fyfe DA, Hagler DJ, Mair DD, et al. Continuous wave Doppler determination of right ventricular pressure: a simultaneous Doppler-catheterization study in 127 patients. J Am Coll Cardiol. 1985;6(4):750-6.

27. Berger M, Haimowitz A, Van Toh A, Berdoff RL, Goldberg E. Quantitative assessment of pulmonary hypertension in patients with tricuspid regurgitation using continuous wave Doppler ultrasound. J Am Coll Cardiol. 1985;6(2):359-65.

28. Lopez-Candales A, Edelman K. Shape of the right ventricular outflow Doppler envelope and severity of pulmonary hypertension. Eur Heart J Cardiovasc Imaging. 2012;13(4):309-16.

29. Kuppahally SS, Michaels AD, Tandar A, Gilbert EM, Litwin SE, Bader FM. Can echocardiographic evaluation of cardipulmonary hemodynamics decrease right heart catheterizations in end-stage heart failure patients awaiting transplantation? Am J Cardiol. 2010;106(11):1657-62.

30. Tamarin R, Torbicki A, Marchandise B, Laaban JP, Morpurgo M. Doppler echocardiographic evaluation of pulmonary artery pressure in chronic obstructive pulmonary disease. A European multicentre study. Working Group on Non-invasive Evaluation of Pulmonary Artery Pressure. European Office of the World Health Organization, Copenhagen. Eur Heart J. 1991;12(2):103-11.

31. Lahm T, Crisostomo PR, Markel TA, Wang M, Weil BR, Novotny NM, et al. The effects of estrogen on pulmonary artery vasoreactivity and hypoxic pulmonary vasoconstriction: potential new clinical implications for an old hormone. Crit Care Med. 2008;36(7):2174-83.

32. Pugh ME, Hennes AR. Development of pulmonary arterial hypertension in women: Interplay of sex hormones and pulmonary vascular disease. Womens Health (Lond Engl). 2010;6(2):285-96.

33. Lahm T, Patel KM, Crisostomo PR, Markel TA, Wang M, Herring C, et al. Endogenous estrogen attenuates pulmonary artery vasoreactivity and acute hypoxic pulmonary vasoconstriction: the effects of sex and menstrual cycle. Am J Physiol Endocrinol Metab. 2007;293(3):E865-71.

34. Lam CS, Borlaug BA, Kane GC, Enders FT, Redfield MM. Age-associated increases in pulmonary artery systolic pressure in the general population. Circulation. 2009;119(20):2663-70.

35. Warnes CA. Pregnancy and pulmonary hypertension. Int J Cardiol. 2004;97 Suppl 1:11-3.

36. Resta TC, Kanagy NL, Walker BR. Estradiol-induced attenuation of pulmonary hypertension is not associated with altered enos expression. Am J Physiol Lung Cell Mol Physiol. 2001;280(1):L88-97.

37. Weiss BM, Zemp L, Seifert B, Hess OM. Outcome of pulmonary vascular disease in pregnancy: A systematic overview from 1978 through 1996. J Am Coll Cardiol. 1998;31(7):1650-7.