Natural History of Pulmonary Hypertension: Adaptive versus Maladaptive Physiologic Responses in Beef Cattle Exposed to Chronic Hypoxia

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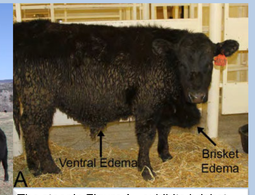
Introduction

1. Humans with group 3 PH characterized by alveolar hypoxia exhibit diversity in disease progression outcomes
2. Similarly certain breeds and individuals among beef cattle, including the American Angus demonstrate variable susceptibility to hypoxia- induced pulmonary vasoconstriction, progressive remodeling of the pulmonary vasculature, pulmonary hypertension (PH) and right heart failure (HF)
3. Despite selection of breeding stock with low PH susceptibility, failure to thrive and death loss associated with PH and right HF remain problematic for unknown reasons
4. Clinical and experimental evidence coupled with gross and microscopic cardiopulmonary lesions suggest inflammation plays a significant role in the pathogenesis of pulmonary vascular remodeling, PH and right HF in cattle and recapitulates features of Group 3 PH in humans

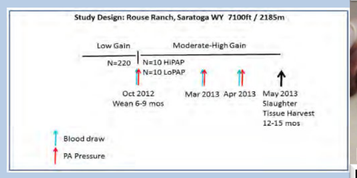
Hypothesis

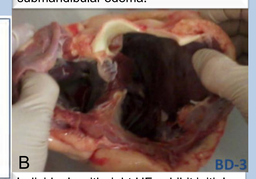
1. The extent of pulmonary inflammation predicts the magnitude of fibroproliferative remodeling in the pulmonary vascular tree and the perivascular adventitia and interstitium of the right ventricle

Methods



The steer in Figure A exhibits brisket edema indicative of right heart failure, historically referred to as "Brisket Disease", "Dropsy" or "Belly Dragger" owing to prominent brisket, ventral and submandibular edema.

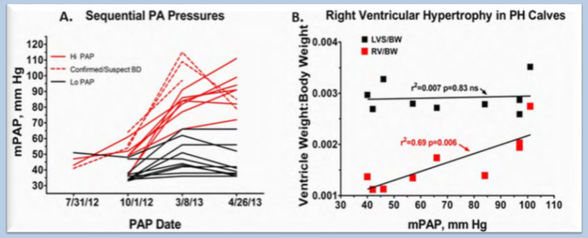


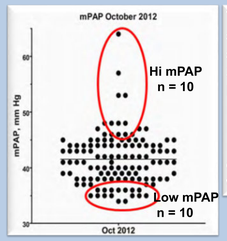


Individuals with right HF exhibit initial compensatory ventricular hypertrophy followed by dilation of the ventricle (decompensation) shown in Figure B.

Jugular vein right heart catheterization and procedures described by Holt and Callan (2007) were used to obtain the mean pulmonary arterial pressures (mPAP) in Angus steers born and raised at the Rouse Ranch. Steers (N = 10 each) were selected at weaning (age 6 mos) with low mPAP (≤38 mm Hg) or high mPAP (≥47 mm Hg). Mean PAP values were re-tested at 12-13 mos age and animals were slaughtered at 15 mos. The total heart weight, isolated right ventricle (RV) and left ventricle (LV) plus septum were recorded in order to calculate ventricle weight:body weight ratios for the right and left ventricles, respectively. Sections of the target cardiopulmonary organs were collected (proximal, middle and distal segments of the right cranial lung lobe, right ventricle, left ventricle, interventricular septum, main pulmonary artery, aorta), fixed in 10% buffered formalin and processed for histopathology. Sections were stained with hematoxylin and eosin and select sections of lung and heart were stained with Masson's Trichrome to demonstrate collagen. The severity of lung and heart lesions was scored semiquantitatively using a grading system developed in consultation with human pathologists to assess pulmonary vascular remodeling, cardiomyocyte hypertophy, interstitial and replacement fibrosis and myofiber disarray associated with PH.

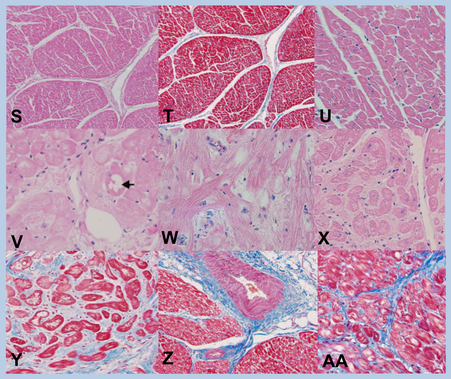
Three Hemodynamic Responses to Chronic Hypoxia





Three distinct phenotypes were identified at slaughter based upon mPAP values and clinical signs: (i) Non-hypertensive (n=10), mPAP range 36-66, median 42 mm Hg; (ii) PH without signs of RV dysfunction (n=7), mPAP 72-111, median 94 mm Hg; (iii) PH with signs of RV failure (n=3), mPAP 79-97 mm Hg. Average RV:body weight ratio was significantly increased for all PH animals compared to non-hypertensive, whereas LV:body weight was unchanged.

Cardiac Histopathology Indicates PH Progression



Low mPAP Steers with No Cardiac Pathologic Alterations

S. Normal right ventricular myocardium

T. Interstitial fibrosis is not present in the right ventricle

U. Right ventricular myocardium with normal cardiomyocytes

High mPAP Steers with Cardiac Lesions

V. Cytoplasmic vacuolization in the right ventricle

W. Myofibrillar disarray in the right ventricular myocardium

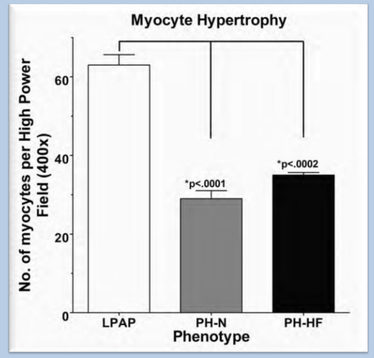
X. Replacement fibrosis in the right ventricular myocardium

Y. Masson's Trichrome highlighting replacement fibrosis

Z. Masson's Trichrome demonstrating thickened adventitial matrix surrounding mural arteries

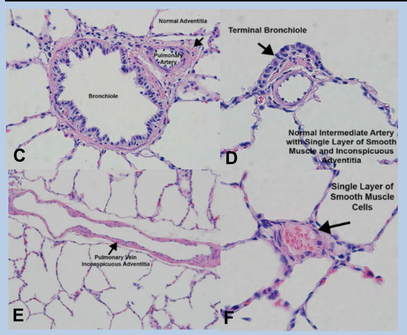
AA. Masson's Trichrome demonstrating entrapped cardiomyocytes

Maladaptive lesions in the left ventricle were not present (Not Shown)



*The average number of cardiomyocytes was determined by counting the number of cells in 10 high power (400X fields) for each ventricle. In order for a cardiomyocyte to be counted, the entire cross sectional area of the cell had to be contained within the boundaries of a 250 x 250 micron field.

Pulmonary Histopathology Indicates PH Progression Influenced by Inflammation



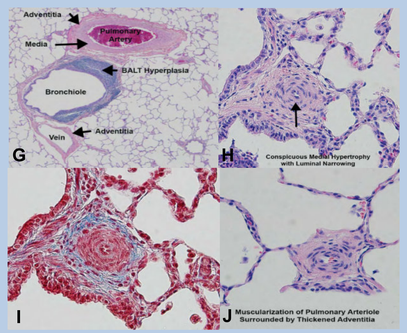
Low mPAP Steers with No Pulmonary Lesions

C. Inconspicuous adventitia

D. No smooth muscle hypertrophy or adventitial proliferation

E. Inconspicuous pulmonary vein

F. Arteriole with normal tunica media and luminal diameter



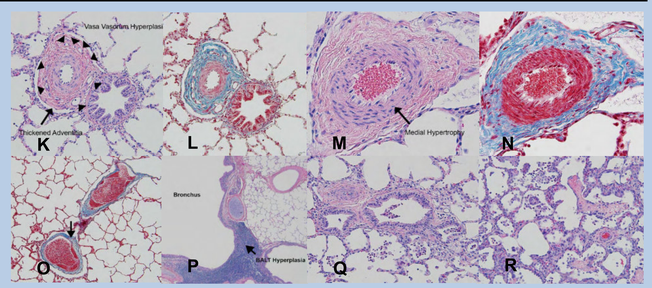
High mPAP Steers with PH

G. Pulmonary artery and vein with mildly thickened adventitia and prominent BALT

H. Conspicuous medial hypertrophy and luminal narrowing

I. Medial hypertrophy and adventitial proliferation highlighted with Masson's Trichrome stain

J. Pulmonary arteriole with prominent muscularization and expansion of the adventitia



High mPAP Steers with PH and Right HF

K. Prominent adventitial thickening and vasa vasorum hyperplasia. The intermediate artery is larger than its associated airway

L. Prominent adventitia and vasa vasorum hyperplasia highlighted with Masson's Trichrome stain

M. Prominent medial hypertrophy and severe adventitial thickening

N. Severe adventitial expansion highlighted with Masson's Trichrome stain

O. Conspicuous pulmonary vein with prominent adventitia highlighted by Masson's Trichrome stain

P. Bronchus with prominent BALT

Q. Alveolar septa and lumina infiltrated and filled by moderate numbers of neutrophils and mononuclear cells

R. Inflammation, alveolar fibrosis, organizing fibrin exudate and hyperplasia

Summary

1. Similar to humans, there is variation among beef breeds and individual steers to alveolar hypoxia
2. Variation in hemodynamics and vascular remodeling is directly correlated to the extent of pulmonary inflammation
3. BALT expands in pulmonary hypertension and may influence pulmonary vascular remodeling and PH
4. Steers with the most severe pulmonary vascular remodeling, PH and right HF have evidence of pulmonary venocclusive disease, a novel finding in this species

Conclusion

Beef cattle raised in chronic hypoxic environments provide a natural model of Group 3 PH where pulmonary inflammation and venous remodeling predict disease severity and outcomes and may provide novel therapeutic targets for the treatment of PH in humans and beef cattle.