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Defining the relationship between female sex hormones and myocardial fibrosis in aortic-banded Yucatan mini-swine

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ABSTRACT

Approximately 50% of heart failure (HF) cases in the United States are diagnosed as heart failure with preserved ejection fraction (HFpEF). HFpEF is diagnosed in women twice as often as in men, with morbidity and mortality rates increasing with age. These sex and age-specific effects point to the involvement of menopause, during which aging women naturally lose ovarian sex hormone production. The purpose of this study was to determine the relationship between HFpEF and ovarian sex hormones on the development of left ventricular (LV) fibrosis in response to chronic pressure-overload. For this study, an ovariectomy (OVX) model of menopause and aortic-banded (AB) model of HFpEF were used in female mini swine divided into four groups: control-intact (CON, n=7), AB-intact (AB, n=7), control-ovariectomized (CON-OVX, n=6), and AB-ovariectomized (AB-OVX, n=7). Total LV collagen protein and mRNA levels of specific collagen (Collagen I & III), matrix metalloproteinase (MMP-2 & -9), and tissue inhibitors of MMP (TIMP-1 & -4) isoforms were measured. Tissue samples from the LV were hydrolyzed in hydrochloric acid and collagen-specific hydroxyproline was quantified using a colorimetric assay and reflected as the ratio of total collagen: total protein. Quantitative RT-PCR was used to measure mRNA levels of extracellular matrix components and their regulatory biomarkers. We hypothesize that AB pigs will have increased total collagen and increased MMP-2, MMP-9, TIMP-1, TIMP-4, Collagen I, and Collagen II mRNA levels when compared to CON. These pathological changes will be exacerbated by the loss of ovarian sex hormones.

OBJECTIVE

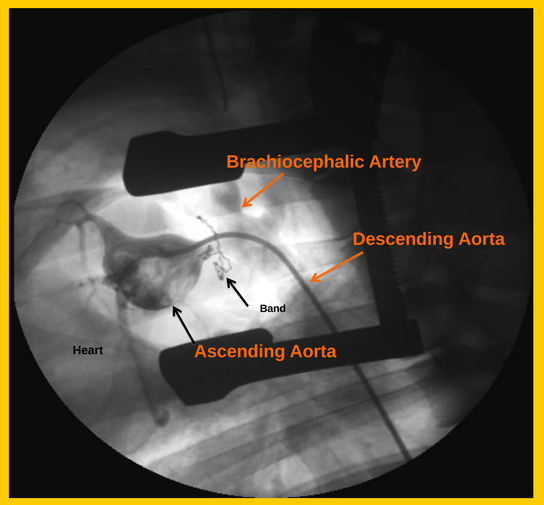
To determine the relationship between HFpEF and ovarian sex hormones on the development of left ventricular (LV) fibrosis in response to chronic pressure-overload.

HYPOTHESIS

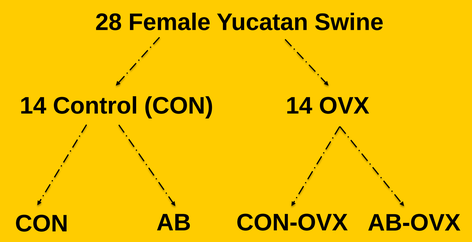
Chronic pressure-overload will increase total collagen and increase MMP-2, MMP-9, TIMP-4, and collagen I mRNA levels. The loss of ovarian sex hormones will exacerbate aortic-banding induced increases in LV collagen deposition.

METHODS

Aortic Band Placement:



A left second space intercostal thoracotomy was performed. A sterile zip tie surrounded by Gore-Tex tubing was placed around the ascending aorta proximal to the brachiocephalic artery. An approximate 70 mm systolic transtenotic gradient (measured using a fluid-filled catheter – femoral artery insertion) was achieved while maintaining a peripheral vascular mean arterial pressure (MAP) of about 90 mm Hg under anesthesia using phenylephrine (I.V. 1-3 ug/kg/min) at a heart rate of about 100 beats/min.



- **Yucatan mini-swine were delivered at 6 mo. old**
- **Ovariectomy (OVX) was performed at 7 mo. old**
- **Aortic-banding (AB) was performed at 8 mo. old**
- **Chronic pressure-overload lasted 6 mo.**
- **Terminal experiments were performed at 14 mo. old**

Total Collagen Assay:

Tissue samples from the LV were hydrolyzed in hydrochloric acid and collagen-specific hydroxyproline was quantified using a colorimetric assay and reflected as the ratio of total collagen: total protein. (Total Collagen Assay, Quickzyme Biosciences)

qRT-PCR Analysis:

Quantitative RT-PCR was used to measure mRNA levels of extracellular matrix components and their regulatory biomarkers. Messenger RNA levels of specific collagen (Collagen I), matrix metalloproteinase (MMP-2 & -9), and tissue inhibitors of MMP (TIMP-4) isoforms were measured.

RESULTS

M.E. - OVX

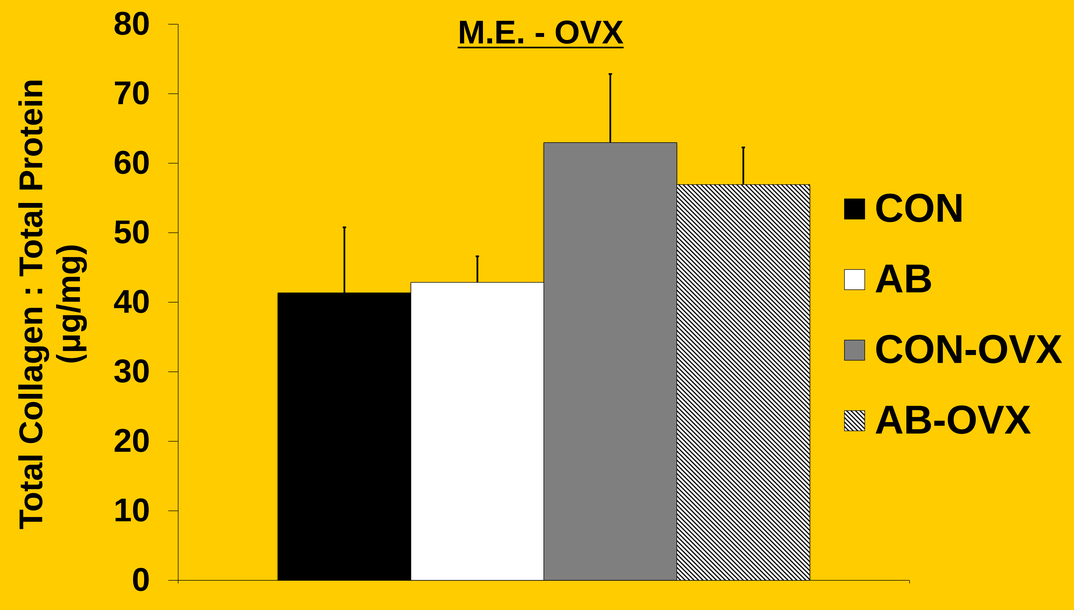


Figure 1. The loss of female sex hormones increases total LV collagen levels (Main Effect – OVX; P < 0.05).

Interaction

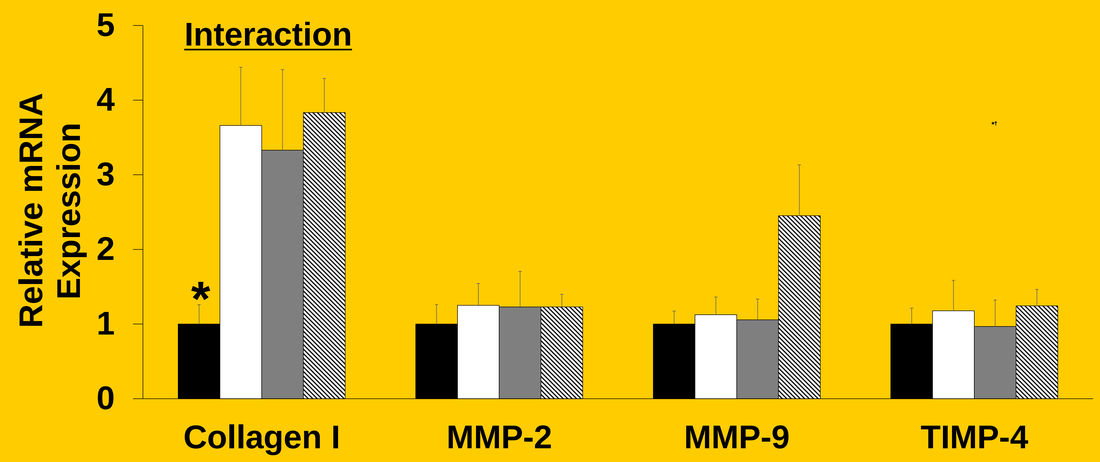


Figure 2. Aortic-banding induced increases in collagen I mRNA expression are dependent on sex hormone status (Interaction; P = 0.09).

CONCLUSIONS

Collagen I mRNA levels were increased by both ovariectomy and chronic pressure-overload, although this only translated to increased total LV collagen protein in ovariectomized animals. Our results suggest menopause can influence the development of heart failure by increasing LV collagen deposition, potentially increasing LV stiffness.

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