Altered testicular vascularization and impaired blood supply in the 41,XX^{Y*} mouse model for Klinefelter syndrome

C. Brand, O. S. Damm, A.-S. Warmeling, R. Sandhowe-Klaverkamp, S. Werler, K. Körner, J. Stypmann[1], M. Kuhlmann[2], R. Holtmeier[2], M. Zitzmann, F. Tüttelmann[3], J. Gromoll & J. Wistuba

Centre of Reproductive Medicine and Andrology, [1]Department of Cardiovascular Medicine, [2]European Institute for Molecular Imaging (EIMI), [3]Institute of Human Genetics, University of Münster, 48149 Münster, Germany

INTRODUCTION

Intratesticular testosterone levels in Klinefelter syndrome (KS) are comparable to controls and Leydig cell function was proven to be normal at least *in vitro* [1]. Therefore, testicular vascularization changes came into focus as a potential factor contributing to hypogonadism [2].

METHODS

We performed enhanced ultrasound based analysis of the testicular blood support in our 41,XX^{Y*} mice (Fig.1). Adult male 41,XX^{Y*} (n=5) and control mice (n=6) underwent ultrasound analyses with the Non-Targeted Contrast Agent Vevo MicroMarker. The agent containing gas filled micro-bubbles was administered intravenously. After initial perfusion, micro-bubbles were destroyed by high ultrasound pressure and the reperfusion period was analysed. In parallel, electrocardiograms (ECGs) were taken. Afterwards mice were sacrificed and testes removed for histological analysis of vascularization.

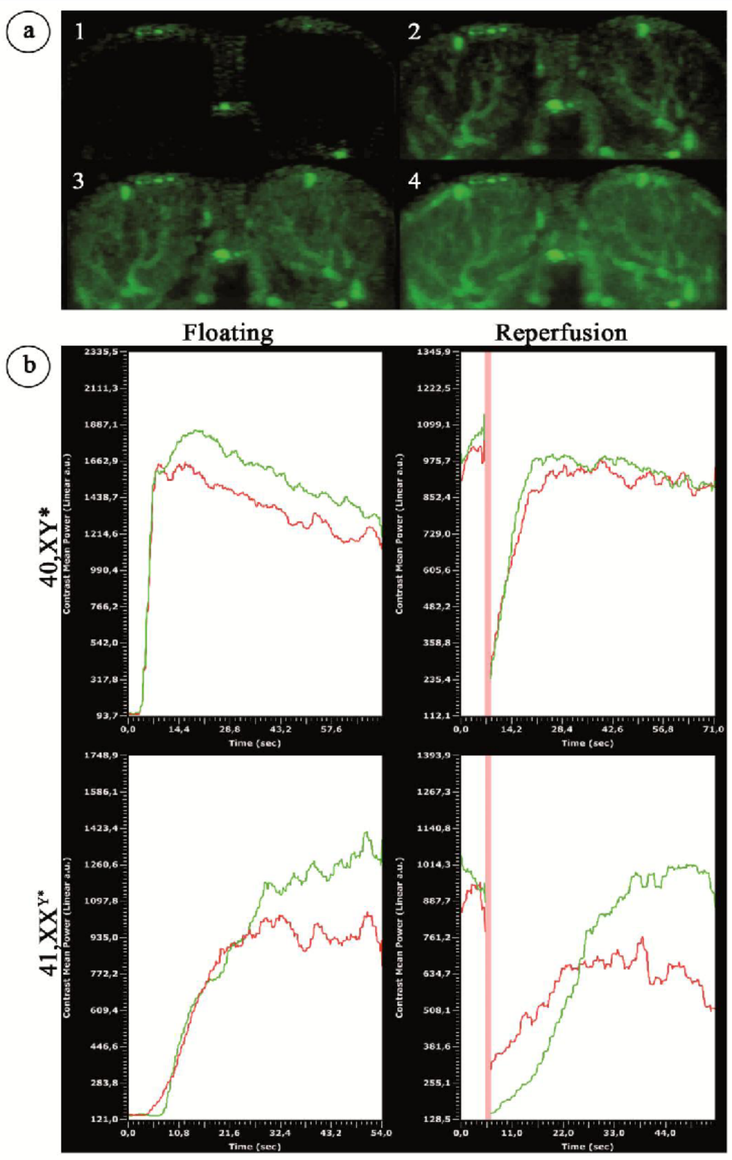

***Figure 1* Contrast-enhanced ultrasound for functional analysis of the testicular blood flow in adult (15 wpp) 40,XY* and 41,XX^{Y*} mice.** To compare the functionality of the testicular vascularization of 40,XY* and 41,XX^{Y*} mice a contrast agent was injected and the filling of the testes over time with this contrast agent was analyzed. (a) Example of the filling of the testes is shown from time point 1 (before the contrast agent entered the testes) over 2 and 3 to 4 (the testes are filled at the maximum with contrast agent). (b) Example of the ultrasound signal intensity measured over time of testis filling is illustrated for 40,XY* and 41,XX^{Y*} mice, respectively. Floating is the first filling of the testes after injection of the contrast agent. After floating, destruction of the contrast agent was induced by transmission of a high power ultrasound pulse before the testes filled again with contrast agent (reperfusion). Green = signal measured for the right testis; Red = signal measured for the left testis.

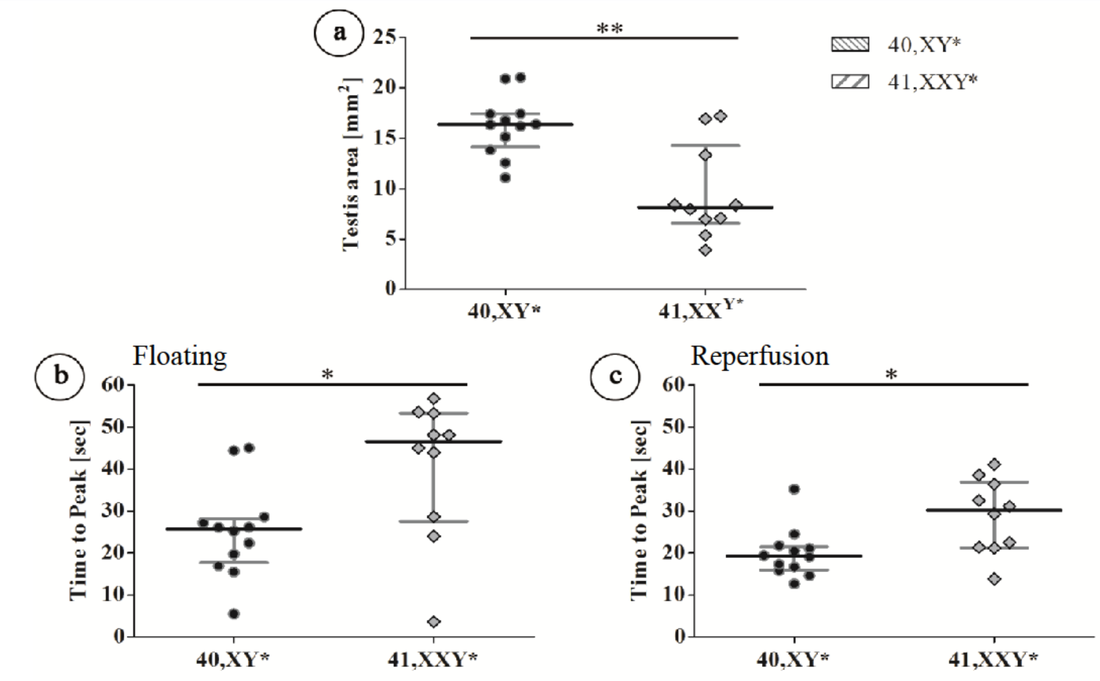

***Figure 2* Analysis of the testicular blood flow in adult (15-16 *wpp*) 40,XY* and 41,XX^{Y*} mice by contrast-enhanced ultrasound.** Filling of the testes with contrast agent was analyzed by ultrasound imaging after agent injection. ECGs were recorded (no significant differences) and the area of the region of interest (ROI; equals testis area) was determined. (a) ROI (b) Time to peak of floating (c) Time to peak of reperfusion. *: p<0.05; **: p<0.01

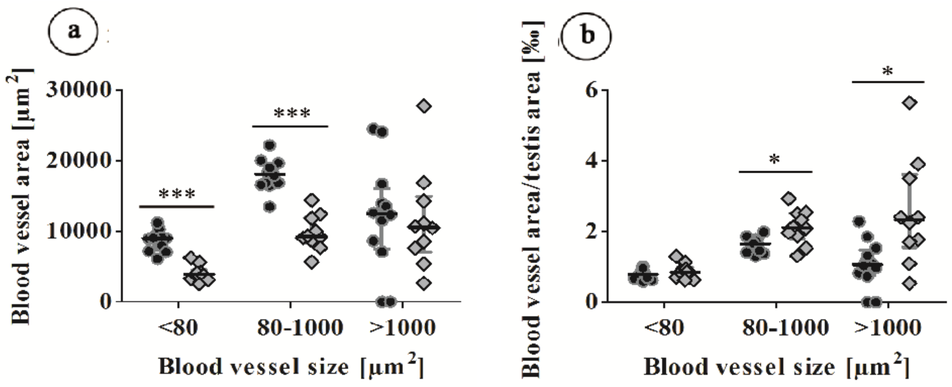

***Figure 3* Blood vessel size distribution in testes of 15 wpp mice of the karyotypes 40,XY* and 41,XX^{Y*}.** Data from histological analysis according to Tüttelmann *et al.* (2014). Blood vessels were categorized according to the area they cover. The areas of all blood vessels belonging to the size categories <80 µm2, 80-1000 µm2 and >1000 µm2 were summed up. The blood vessel area/testis area ratio was determined considering always only vessels of the respective size categories. (a) testicular area covered by vessels belonging to the three size categories (b) Testicular blood vessel area/testis area ratio considering vessels of three size categories. All data are presented as median values with interquartile ranges. Dotplots additionally include the individual values obtained from each testis analyzed. *: p<0.05; **: p<0.01; ***: p<0.001

RESULTS

Whilst ECGs did not reveal differences in heart function, the reperfusion time for testes was significantly increased in 41,XX^{Y*} mice (XX^{Y*} 28.8± 1.7s; XY* 19.9± 2.8s, Fig.2c). Testes of 41,XX^{Y*} mice (Fig.2a) and the area covered by blood vessels (XX^{Y*} 0.025 ± 0.003mm²; XY* 0.040 ± 0.002mm², Fig.3a) were significantly smaller. Testicular blood vessel areas of adult males were assigned to four categories (I=<80µm², II=80-1000µm², III=1000-5062µm², V=>5062µm², Fig.3). The blood vessel area of categories I and II was significantly decreased in 41,XX^{Y*} mice (p<0.0001). Taking the testis area into account, the area covered by vessels of category II and III is significantly elevated in KS mice. Blood vessels of category IV were missing in KS testes.

CONCLUSIONS

These functional and morphological data strengthen the assumption that the observation made previously contributes to the endocrine phenotype seen in KS pointing to an affected vascular system in the disturbed testicular tissue of males with supernumerary X.

References

[1] Wistuba et al., Endocrinology. 2010;151(6):2898-910.
[2] Tüttelmann et al., Andrology. 2014;2(2):275-81.

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