Charcoal-stripped serum impairs growth and sprouting of male and female human umbilical vein endothelial cells (HUVECs) regardless of estrogens

C. Vanetti[1], E. Cappellini[2], L.M. Vicentini[1], M.G. Cattaneo[1]

[1]Dept of Medical Biotechnology and Translational Medicine, Pharmacology Unit, University of Milan, Italy
[2]Division of Auxology and Metabolic Diseases, Istituto Auxologico Italiano, IRCCS, Verbania, Italy

Loss of a normal endothelial function crucially contributes to the development and clinical expression of atherosclerosis and cardiovascular disease (CVD). Despite substantial sex/gender differences have been described in the occurrence of CVD, very little is known about innate sex properties of male and female endothelial cells (ECs).

To characterize putative sex-dependent differences, we compared *in vitro* properties of male and female human umbilical vein ECs (HUVECs). Importantly, we always used HUVECs pooled from two or more donors to minimize the variability associated with cells derived from a single male or female newborn donor. We have previously shown that female HUVECs express an higher amount of endothelial Nitric Oxide Synthase (eNOS) mRNA and protein, and possess greater migratory capabilities in comparison to male cells[1]. On the other hand, male and female HUVECs did not show any difference when metabolic activity was compared.

A crucial issue in endothelial patho-physiology concerns estrogens and their involvement in the sex-related incidence of CVD. To study the contribution of estrogens to EC physiology, we compared metabolic properties of male and female HUVECs grown in standard medium (SM) or in a nominally estrogen-free medium (199 medium w/out phenol red supplemented with charcoal-stripped FBS, ChM). We found that lack of hormones induced a significant decrease in cell number, and consequently in metabolic activity (evaluated by MTT and ATP), in comparison to standard growth conditions. The loss in cell number did not depend on EC apoptosis and/or necrosis, and was super imposable between male and female ECs. However, the addition of 17β-estradiol (E2) did not revert the decreases in cell number and metabolic activity in both male and female ECs when cultured in ChM. Similarly, the addition of phenol red alone or in combination with E2 left unaffected both male and female metabolic activity. These results suggest that estrogens did not represent a critical factor for the maintenance of metabolic activity in male and female ECs cultured in an hormone-free medium. Super imposable results were obtained when cells were cultured in 199 medium w/phenol red supplemented with charcoal-stripped FBS (ChM w/phenol red). Furthermore, *in vitro* angiogenesis (evaluated by a 3-D spheroid sprouting assay) was significantly impaired in ChM w/phenol red, suggesting that EC growth and sprouting critically require some serum components that are lost in charcoal-stripped FBS. Searching for valuable candidates, we focused our attention on fatty acids (FAs) given that charcoal-stripped FBS is fully depleted from them. Preliminary results favor the hypothesis of a role for FAs in EC rescue from charcoal-stripped FBS-impaired metabolic activity.

In conclusion, our results suggest that one or more substances removed from FBS by stripping procedure and different from E2 are crucial for the maintenance of both male and female HUVEC behavior. Among these substances, FAs might act as fundamental regulators of EC growth and sprouting.

[1]Addis R, Campesi I, Fois M, Capobianco G, Dessole S, Fenu G, Montella A, Cattaneo MG, Vicentini LM, Franconi F. Human umbilical endothelial cells (HUVECs) have a sex: characterisation of the phenotype of male and female cells. Biol Sex Differ. 2014 5(1):18. doi: 10.1186/s13293-014-0018-2.