

## **FATTY ACIDS RESTORE IN VITRO ANGIOGENIC PROPERTIES IN HUMAN MALE AND FEMALE ENDOTHELIAL CELLS CULTURED IN HORMONE-DEPRIVED SERUM**

1)Vanetti C. 2)Bifari F. 3)Vicentini LM. 4)Cattaneo MG.

Hormone-deprived serum (charcoal-stripped serum, CSS) is a well-accepted method to model effects of sex hormones in cell cultures. We have recently shown that CSS decreases human endothelial cell (EC) growth and in vitro angiogenesis even when estrogen concentration is restored<sup>1</sup>. However, whether male or female ECs are similarly affected by CSS is unknown. Moreover, the mechanism(s) underlying the CSS-induced decrease in EC in vitro functions remains to be determined. In this study, we independently studied male and female ECs and found that CSS inhibited growth and angiogenesis in cells from both sexes, with a more pronounced effect on male ECs. We further assessed whether 17- $\beta$  estradiol (E2) and dihydrotestosterone (DHT) deprivation may have a sex-dependent effect on male and female ECs cultured with CSS. Despite the effect of sex hormones on EC biology has been reported in literature, our results showed that E2 and DHT did not significantly revert the CSS-induced phenotype in ECs of both sexes. Again, the addition of the lipophilic thyroid hormone, also depleted in CSS-containing medium, did not prevent the CSS-induced male and female EC inhibition. Therefore, we further investigated other essential metabolites lost in CSS and able to rescue proliferative and angiogenic properties of male and female ECs cultured with this serum. We found that supplementation with the fatty acid (FA) palmitic acid or the acetyl-CoA precursor acetate significantly rescued the CSS-induced inhibition of growth and sprouting in ECs of both sexes. Therefore, the loss of metabolic precursors (e.g., FAs) rather than hormones is involved in the impairment of in vitro proliferative and angiogenic properties of male and female ECs cultured with CSS.

In conclusion, we identify the lack of FAs as responsible, at least in part, for the profound CSS-induced alterations in EC behavior. Our results suggest that part of the phenotype observed in human primary cell cultures or cell lines cultured with CSS may result from its deficiency in essential metabolites (e.g., FAs). Consequently, biological effects of hormone might be affected by the concurrent loss of essential metabolites, and we could observe some metabolic rather than endocrine responses in CSS-cultured ECs. For that reason, it is important to have knowledge of the undervalued contribute that CSS may give to experimental outcomes from in vitro experiments and to their interpretation.

<sup>1</sup>Vanetti et al (2016) *Endocr Res* 41, 325-333.