Clinical evidence suggests that the natural estrogen estetrol (E4) is associated with a neutral impact of coagulation due to minimal liver effects, as well as to potential endothelial protective actions. We explored the modulatory action of E4 and estradiol (E2) on the plasminogen activation pathway (PAI-1, u-PA and t-PA) in human umbilical vein endothelial cells (HUVEC). Western analysis indicates that E4 effect is profoundly different as compared to E2. When provided to HUVEC, E4 does not turn into significant changes in the synthesis and release of PAI-1 or u-PA. In addition, when used in co-treatment with E2, E4 blocks the stimulatory effect of E2 on PAI-1, with an overall effect that is related to the E2/E4 concentration ratio. Similar patterns were identified when analyzing the effects of E4, E2 or the combination on the expression and release of u-PA. These findings corroborate the concept of the diversity of E4 vs. E2 related to the ability to control endothelial cells. The effects identified in this study may be related to the safer profile on vein thrombotic events that has been reported for E4.