Estrogens play an important role in endometrial cancer (EC). The majority of EC cases arise in postmenopausal women when ovaries cease to produce estrogens, so they are only synthesized from adrenal steroid precursors at peripheral sites, such as breast, bone, adipose tissue, and also endometrium. In these tissues estrogens can be formed via the aromatase pathway from androgens, or via the sulfatase pathway from estrone sulfate (E1S). Three enzymes have been postulated as crucial for the synthesis of estradiol (E2), aromatase (CYP19A1), 17ß-HSD type 1 (HSD17B1), and sulfatase (STS), and are already considered as potential drug targets. But despite the numerous studies, the contributions of the aromatase and the sulfatase pathways in local production of E2 in EC have not yet been unambiguously determined. We investigated the local formation of E2 in paired cancerous and adjacent control endometrium samples. First, we confirmed that E2 and its precursor androstenedione (A-dione) are indeed present in EC and control tissues. Then, we investigated the expression of 15 genes of the aromatase and sulfatase pathways. While most genes encoding biosynthesizing enzymes were well expressed in EC, the mRNA levels of HSD17B1 and CYP19A1 were extremely low. Finally, we evaluated the metabolism of A-dione, E1S and E1 in EC. A-dione was metabolized to testosterone, probably by AKR1C3, but not to E1 or E2. On the other hand, E1S and E1 were metabolized to E2, demonstrating that sulfatase pathway is not only functional but also dominates over the aromatase pathway in EC. Although, aromatase pathway seems to have a marginal role in EC tissues, it is still important for biosynthesis of estrogens in adipose tissue and consequently for elevated E1S blood levels in EC patients.