Characterization of eight types of 17β-hydroxysteroid dehydrogenases from the Japanese sardine Sardinops melanostictus: The probable role of type 12a in ovarian estradiol synthesis

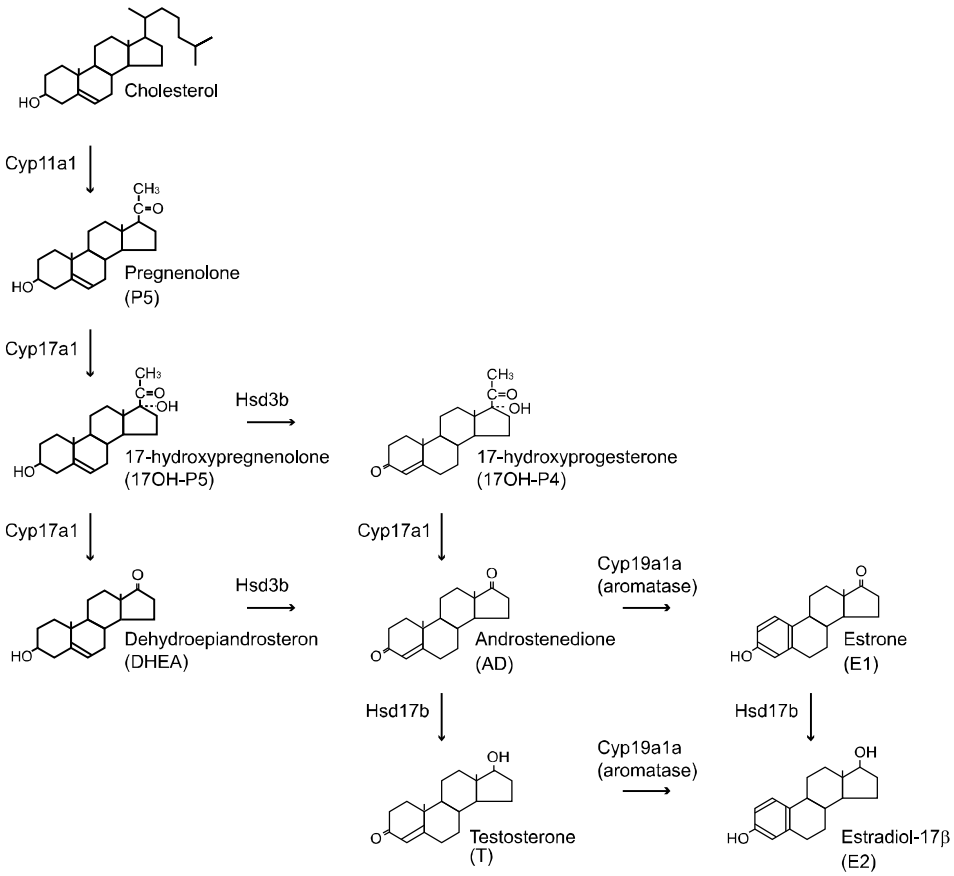

Figure 1

Steroidogenic pathway of the biosynthesis of estradiol-17β (E2) in teleost ovarian follicles. During vitellogenesis, E2 is synthesized from pregnenolone (P5) via 17-hydroxypregnenolone (17OH-P5), 17-hydroxyprogesterone (17OH-P4) or dehydroepiandrosterone (DHEA), androstenedione (AD), and testosterone (T) in chub mackerel Scomber japonicus and Japanese amberjack Seriola quinqueradiata, whereas it is synthesized via AD and estrone (E1) in bambooleaf wrasse Pseudolabrus sieboldi and red seabream Pagrus major. Steroid conversions are catalyzed by enzymes belonging to the cytochrome P450 (CYP) and hydroxysteroid dehydrogenase (HSD) families.

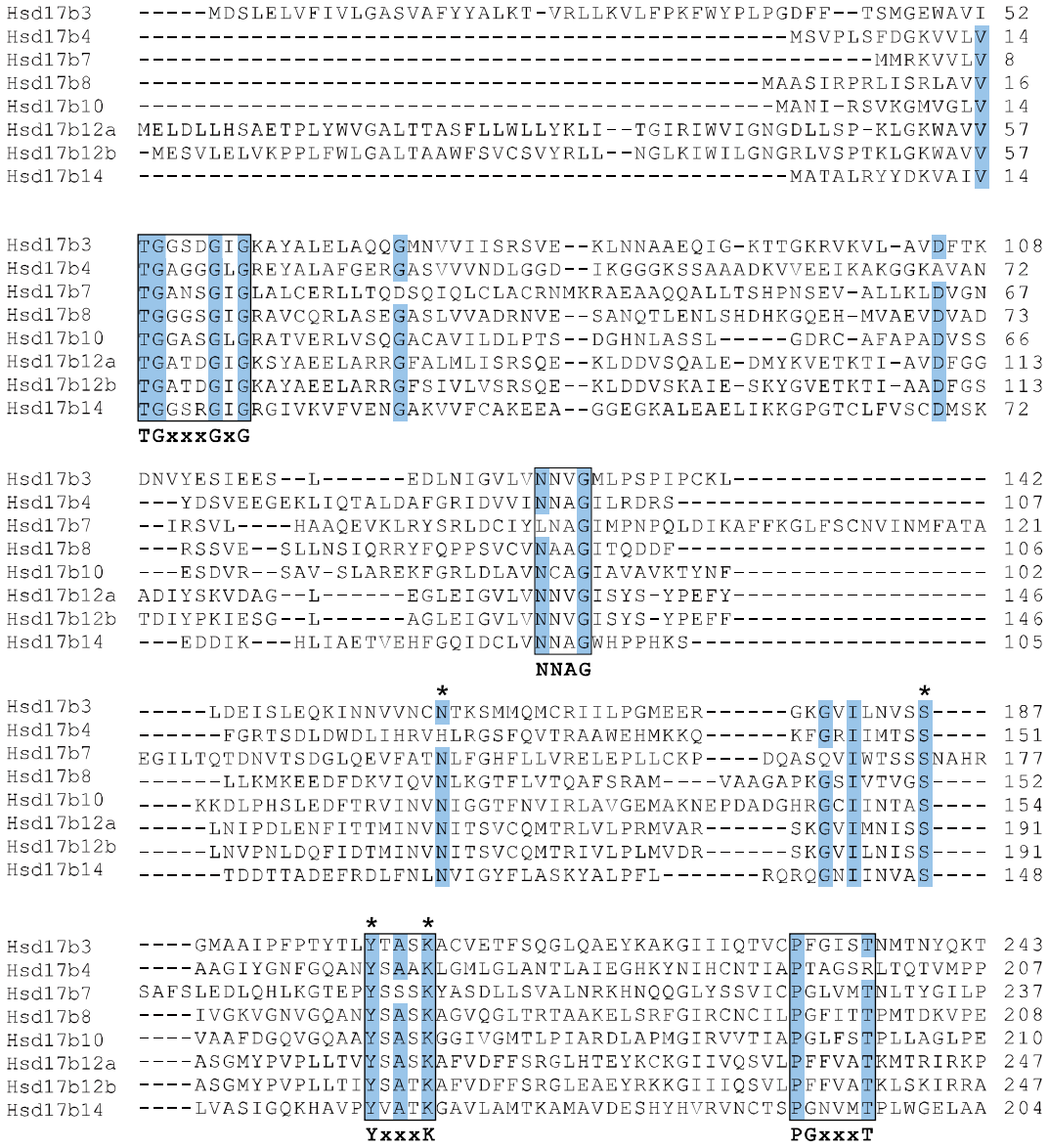

Figure 2

Sequence alignment of the amino acid sequences of eight types of 17 β-hydroxysteroid dehydrogenases (Hsd17b) in the Japanese sardine Sardinops melanostictus. Identical residues are shaded blue; several highly conserved motifs characteristic of the short-chain dehydrogenase/reductase (SDR) superfamily are labeled and indicated by black boxes. Asterisks mark the catalytic tetrad (N-S-Y-K). Numbers specify the number of amino acids.

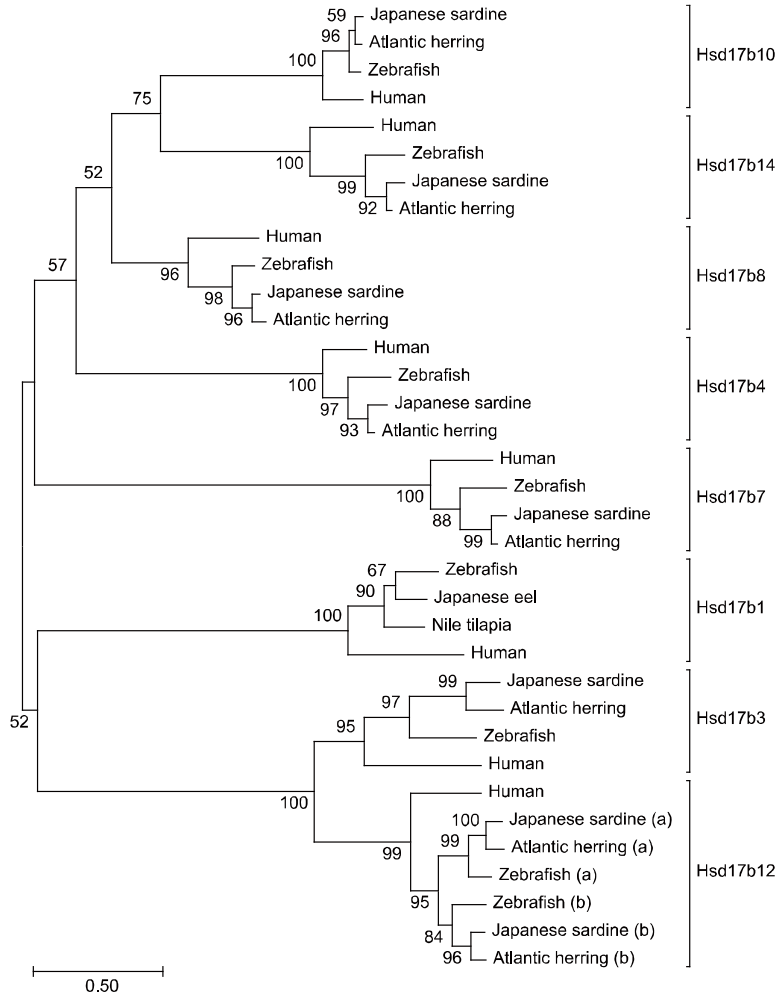

Figure 3

Phylogenetic tree of the amino acid sequences of Hsd17b. Bootstrap analysis was performed using the neighbor-joining method (1,000 replications). GenBank accession numbers of Japanese sardine and Atlantic herring *Clupea harengus* Hsd17b types are shown in Table 1. GenBank accession numbers of zebrafish *Danio rerio* and human Hsd17b/HSD17B types 1, 3, 4, 7, 8, 10, 12, and 14 are as follows: zebrafish, NM_205584, AY551081, AF241285, NM_001077328, NM_001005292, NM_001006098, NM_200881, NM_199613, NM_001003521; human, BC111935, NM_000197, BC003098, BT007075, BC008185, BC000372, BC012043, NM_016246. GenBank accession numbers of Japanese eel *Anguilla japonica* and Nile tilapia *Oreochromis niloticus* Hsd17b1 are AY498620 and NP_001266724, respectively.

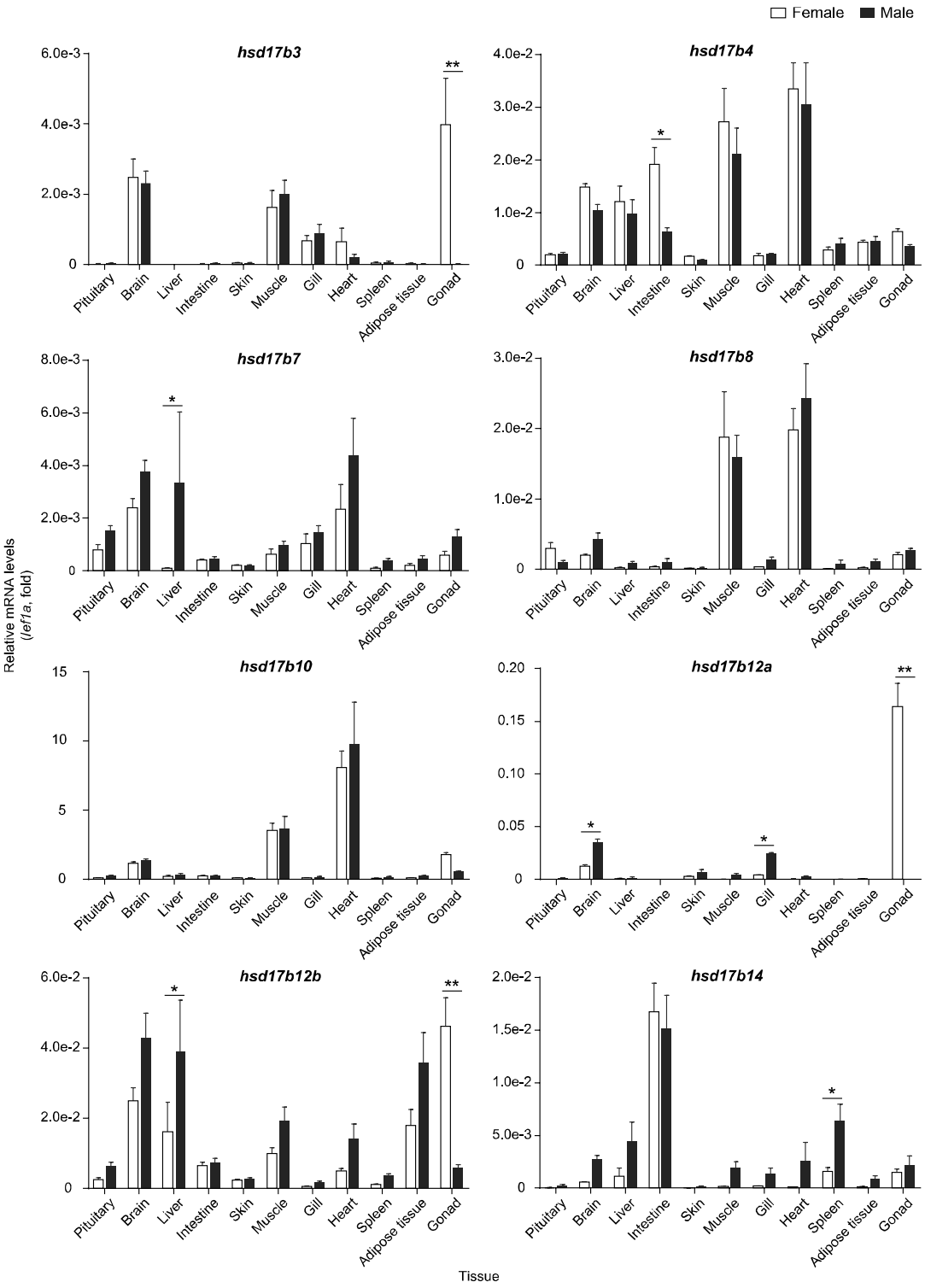

Figure 4

Relative mRNA levels of eight types of Hsd17b distributed in various tissues of the Japanese sardine. Values are the mean ± SEM (n = 4). Asterisks indicate significant differences (*, $p < 0.05$; **, $p < 0.0001$).

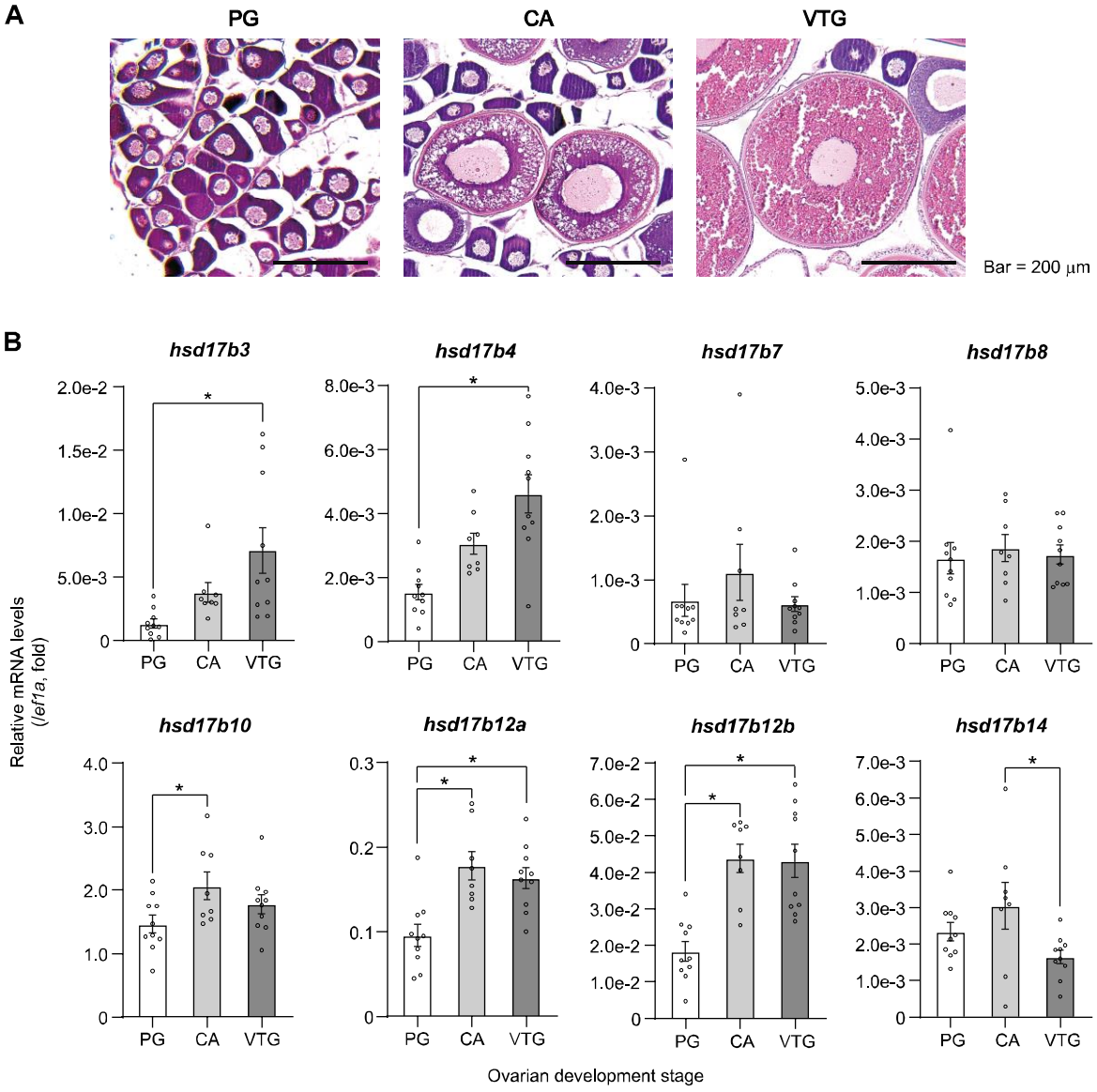

Figure 5

Ovarian Hsd17b gene expression at different developmental stages in the Japanese sardine. (A) Histological micrographs of ovaries at different developmental stages: primary growth (PG), cortical alveolus (CA), and vitellogenic (VTG) stages. (B) Relative mRNA levels of Hsd17b types in sardine ovaries. Values are the mean ± SEM (PG and VTG, n = 10; CA, n = 8). Asterisks indicate significant differences ($p < 0.05$).

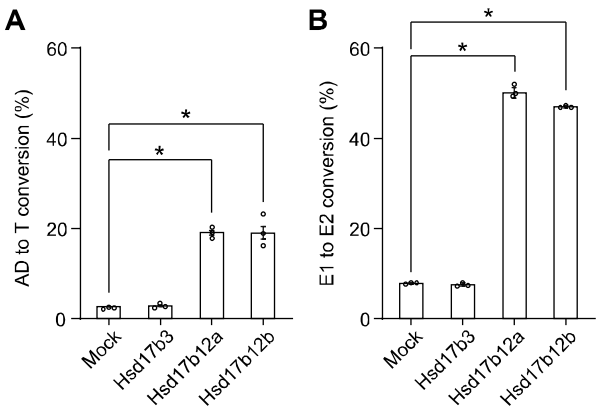

Figure 6

Enzymatic activities of Japanese sardine Hsd17b3, Hsd17b12a, and Hsd17b12b. The conversions of AD to T (A), and E1 to E2 (B), were measured in human embryonic kidney 293T (HEK293T) cells transfected with each Hsd17b expression vector or an empty vector (mock). Values are the mean ± SEM of three replicates. Asterisks indicate significant differences ($p < 0.05$) between cells transfected with empty vector (mock) and each Hsd17b expression vector.

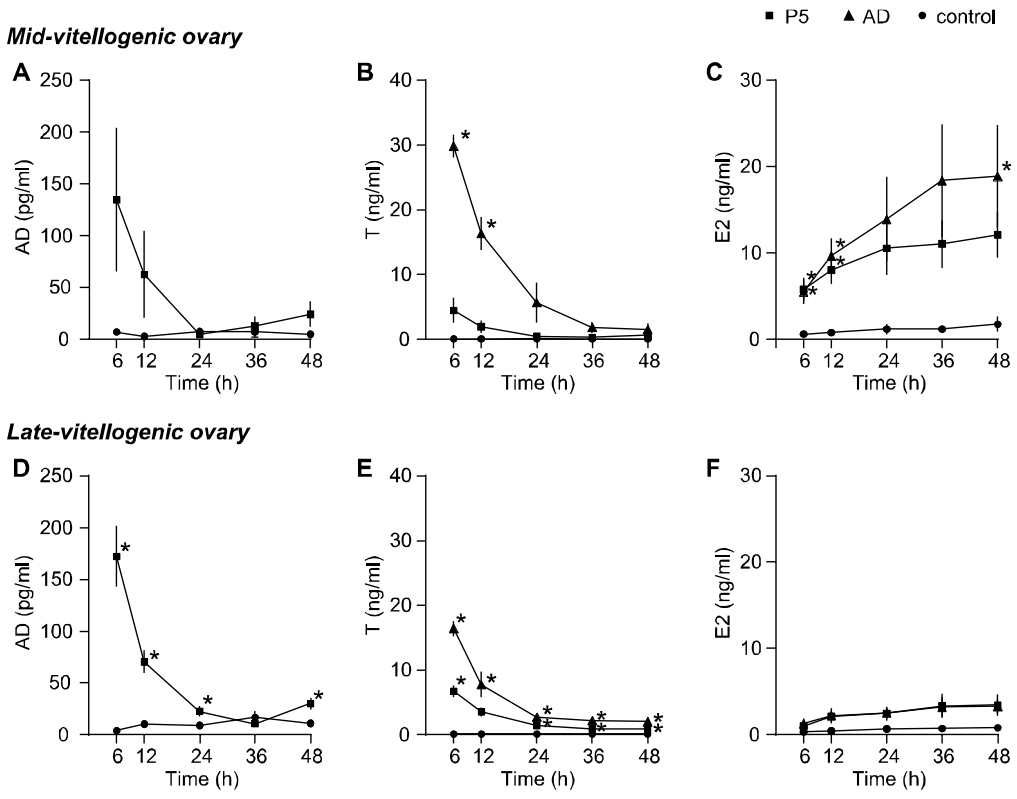

Figure 7

In vitro steroid production of AD, T, and E2 in Japanese sardine ovaries cultured with the steroid precursors, P5 or AD. The ovaries were divided into two groups: mid-vitellogenic ovaries (n = 3; A–C) and late-vitellogenic ovaries (n = 6; D–F). The concentrations of AD (A and D), T (B and E), and E2 (C and F) in the ovary cell culture medium were measured at each time point (6, 12, 24, 36, and 48 h post-incubation). Asterisks indicate significant differences ($p < 0.05$) between the mediums with and without (control) steroid precursors at each sampling point.