

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

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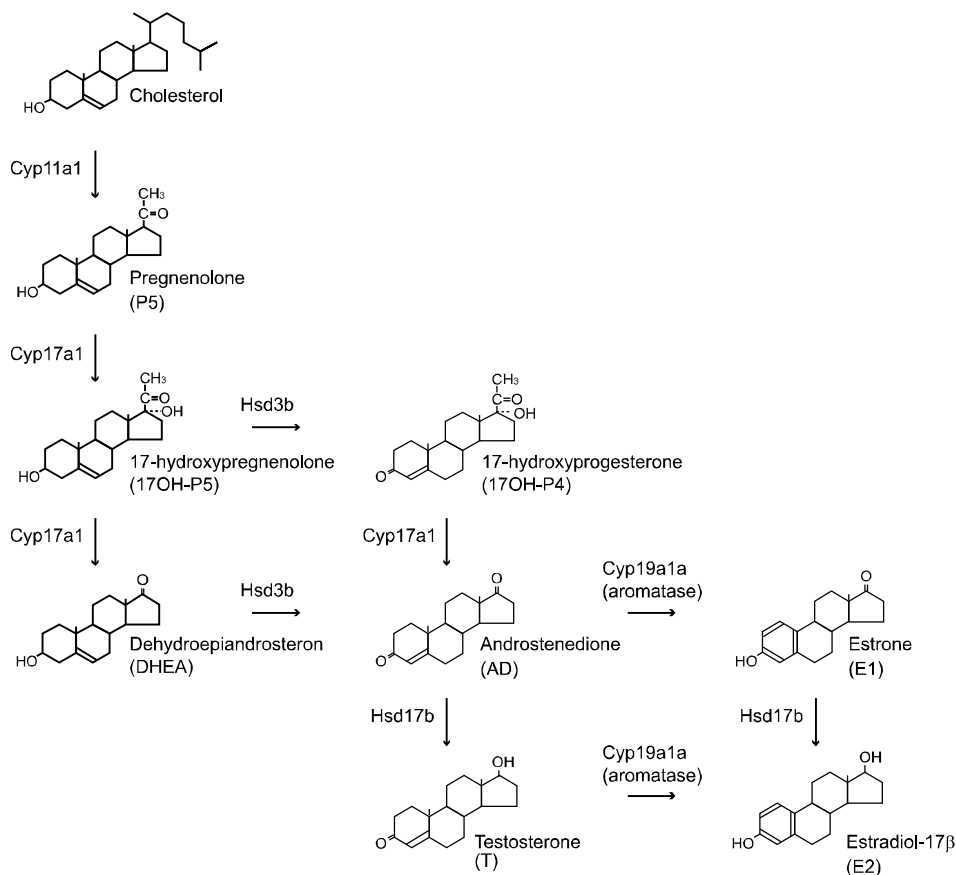


Figure 1

Steroidogenic pathway of the biosynthesis of estradiol-17 β (E2) in teleost ovarian follicles. During vitellogenesis, E2 is synthesized from pregnenolone (P5) via 17-hydroxypregnenolone (17OH-P5), 17-hydroxyprogesterone (17OH-P4) or dehydroepiandrosteron (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.

Hsd17b3	-----MDSLELVFIVLGASVAFYYALKT-VRLKLVLPKFWYPLPGDFF--TSMGEWAVI	52
Hsd17b4	-----MSVPLSFDGKVVLV	14
Hsd17b7	-----MMRKVVLV	8
Hsd17b8	-----MAASIRPRLISRLAVV	16
Hsd17b10	-----MANI-RSVKGMVGLV	14
Hsd17b12a	MELDLLHSAETPLYVWGALTASFLWLKLI--TGIRIWWVIGNGDLLSP-KLGKQAVV	57
Hsd17b12b	-MESVLELVKPPFLFWLGALTAAWFSVCSVYRL--NGLKIWILGNRLVSPTKLGKQAVV	57
Hsd17b14	-----MATALRYDYDKVAIV	14
Hsd17b3	TGGSDGIGKAYALELAQQGMNVVVISRSVE--KLNNAAEQIG-KTTGKRVKVL-AVDFTK	108
Hsd17b4	TGAGGGLGREYALAFGERGASVVVNDLGGD--IKGGGKSSAAADKVVEEIKAKGGKAVAN	72
Hsd17b7	TGANSIGLALCERLLTQDSQIQCLACRNMKRAEAAQQALLTSPNSEV-ALLKLDVGN	67
Hsd17b8	TGGGSGIGRAVCQRLASEGASLVVADRNV--SANQTLENLSHDHKGQEH-MVAEVDVAD	73
Hsd17b10	TGGASGLGRATVERLVSQGACAVILDLPST--DGHNLASSL-----GDRC-AFAPADVSS	66
Hsd17b12a	TGATDGIGKSYAEELARRGFALMLISRSQE--KLDDVSQALE-DMYKVETKTI-AVDFFG	113
Hsd17b12b	TGATDGIGKAYAEELARRGFSIVLVSRSE--KLDDVSKAIE-SKYGVETKTI-AADFGS	113
Hsd17b14	TGGSRGIGRGIVKVFVENGAKVVFCAKEEA--GGEGKALEAELIKKPGTCLFVSCDMSK	72
TGxxxGxG		
Hsd17b3	DNVYESIEES--L-----EDLNIGVLVNNVGMPLSPPIPCKL-----	142
Hsd17b4	---YDSVEEGEKLIQTALDAFGRIDVVIINNAGILRDRS-----	107
Hsd17b7	--IRSVL---HAAQEVKLRYSRLDICIYLNAGIMPNPQLDIKAFKGLFSCNVINMFATA	121
Hsd17b8	---RSSVE--SLNLSIQRRYFQPPSVCVNAAGITQDDF-----	106
Hsd17b10	---ESDVR--SAV-SLAREKFGRLDLAVNCAGIAVAVKTYNF-----	102
Hsd17b12a	ADIYSKVDAG--L-----EGLEIGVLVNNVGISYS-YPEFY-----	146
Hsd17b12b	TDIYPKIESG--L-----AGLEIGVLVNNVGISYS-YPEFF-----	146
Hsd17b14	---EDDIK---HLIAETVEHFGQIDCLVNNAGWNHPPHKS-----	105
NNAG		
Hsd17b3	-----LDEISLEQKINNVCNNTKSMQMCRILPGMEER-----GKGVILNVSS*----	187
Hsd17b4	-----FGRITDLDWDLIHRVHLRGSFQVTRAWEHMKKQ-----KFGRIMTSS----	151
Hsd17b7	EGILTQTDNVTSDGLQEVFATNLFHGHLVRELEPLLCKP-----DQASQVIWTSSSNAHR	177
Hsd17b8	-----LLKMKEEDFDKVIQVNLKGTFLVTQAFSRAM---VAAGAPKGSIVTVGS----	152
Hsd17b10	-----KKDLPHSLEDFTRVINVNIGGTFNVIRLAVGEMAKNEPDADGHRGCIINTAS----	154
Hsd17b12a	-----LNIPDLENFITTMINVNITSVCQMTRLVLPRLMVAR-----SKGVIMNIS----	191
Hsd17b12b	-----LNVPNLDQFIDTMINVNITSVCQMTRIVLPLMVDR-----SKGVILNIS----	191
Hsd17b14	-----TDDTTADEFRDLFNLNVIGYFLASKYALPFL-----RQRQGNINIVAS----	148
Hsd17b3	----GMAAIPFPTYTL*Y*ASKACVETFSQGLQAEYKAKGIIITQVCPFGISTNMTNYQKT	243
Hsd17b4	----AAGIYGNFGQANYSAAKLCMLGLANTLAIEGHKYNHCNTIAPTAGSRLTQTVMP	207
Hsd17b7	SAFSLLEDLQHLKGTEPYSSSKYASDLLSVALNRKHNQQGLYSSVICPGLVMTNLTYGILP	237
Hsd17b8	----IVGKGVNVGQANYSASKAGVQGLTRTAAKELSRFGIRCNCILPFGFITPMTDKVPE	208
Hsd17b10	----VAAFDGQVGQAAYSASKGGIVGMTLPPIARDLAPMGIRVVTIAPGLFSTPLLGLPE	210
Hsd17b12a	----ASGMYPVPLLTVYSASKAFVDFFSRGLHTEYKCKGIIIVQSVLPFFVATKMTIRKP	247
Hsd17b12b	----ASGMYPVPLLTVYSATKAFVDFFSRGLAAYRKKGIIIVQSVLPFFVATKLSKIRRA	247
Hsd17b14	----LVASIGQKHAVPYVATKGAVLAMTKAMAVDESHYHVRVNCSTPGNVMTPLWGELAA	204
YxxxKPGxxxT		

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.

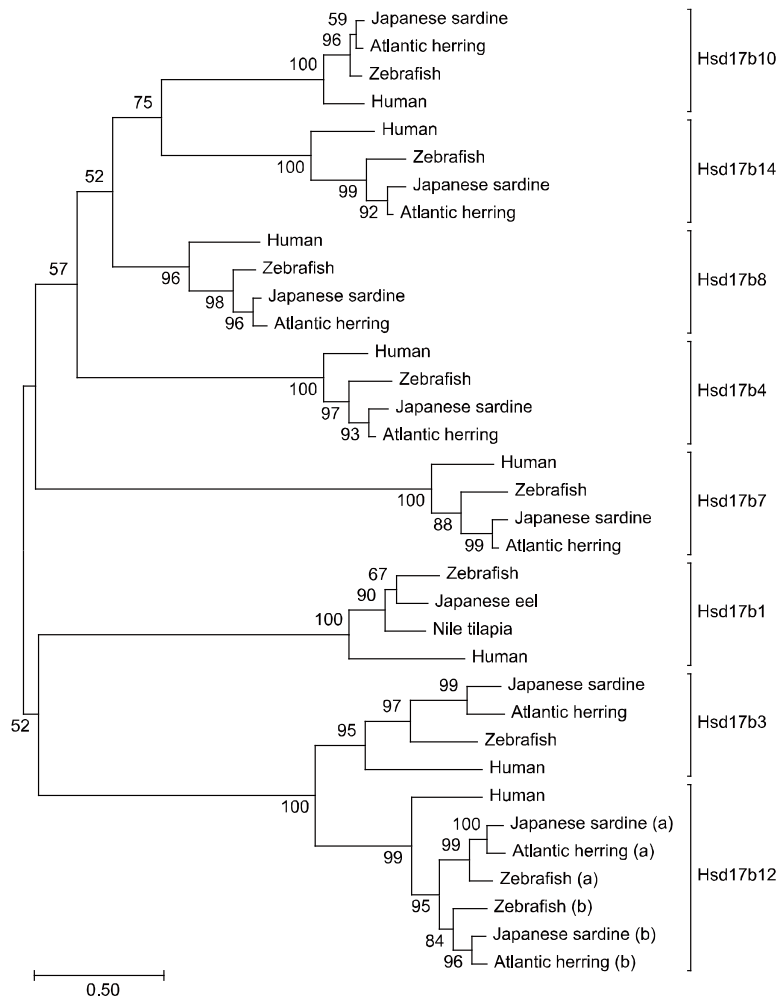


Figure 3

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.

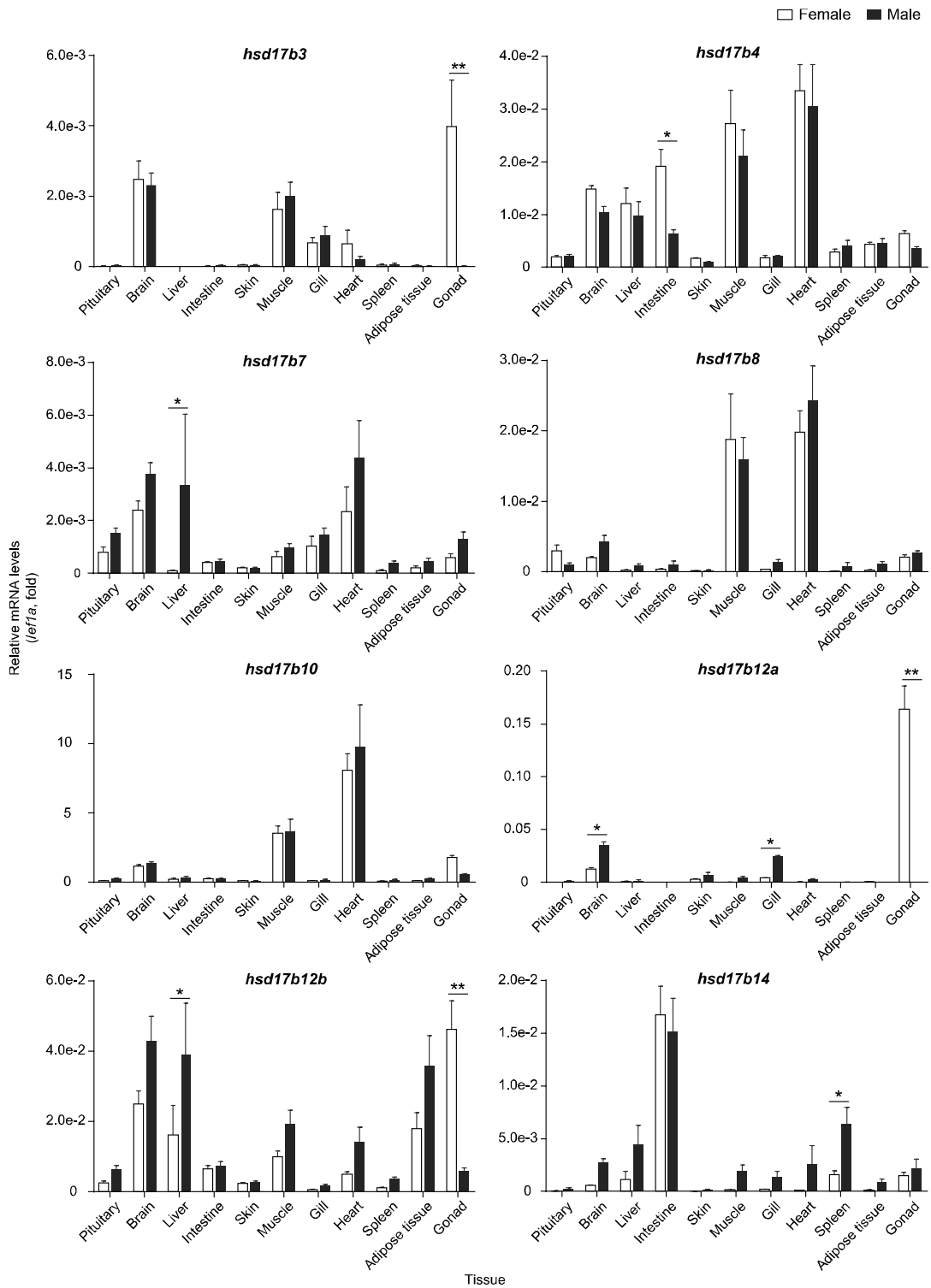


Figure 4

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

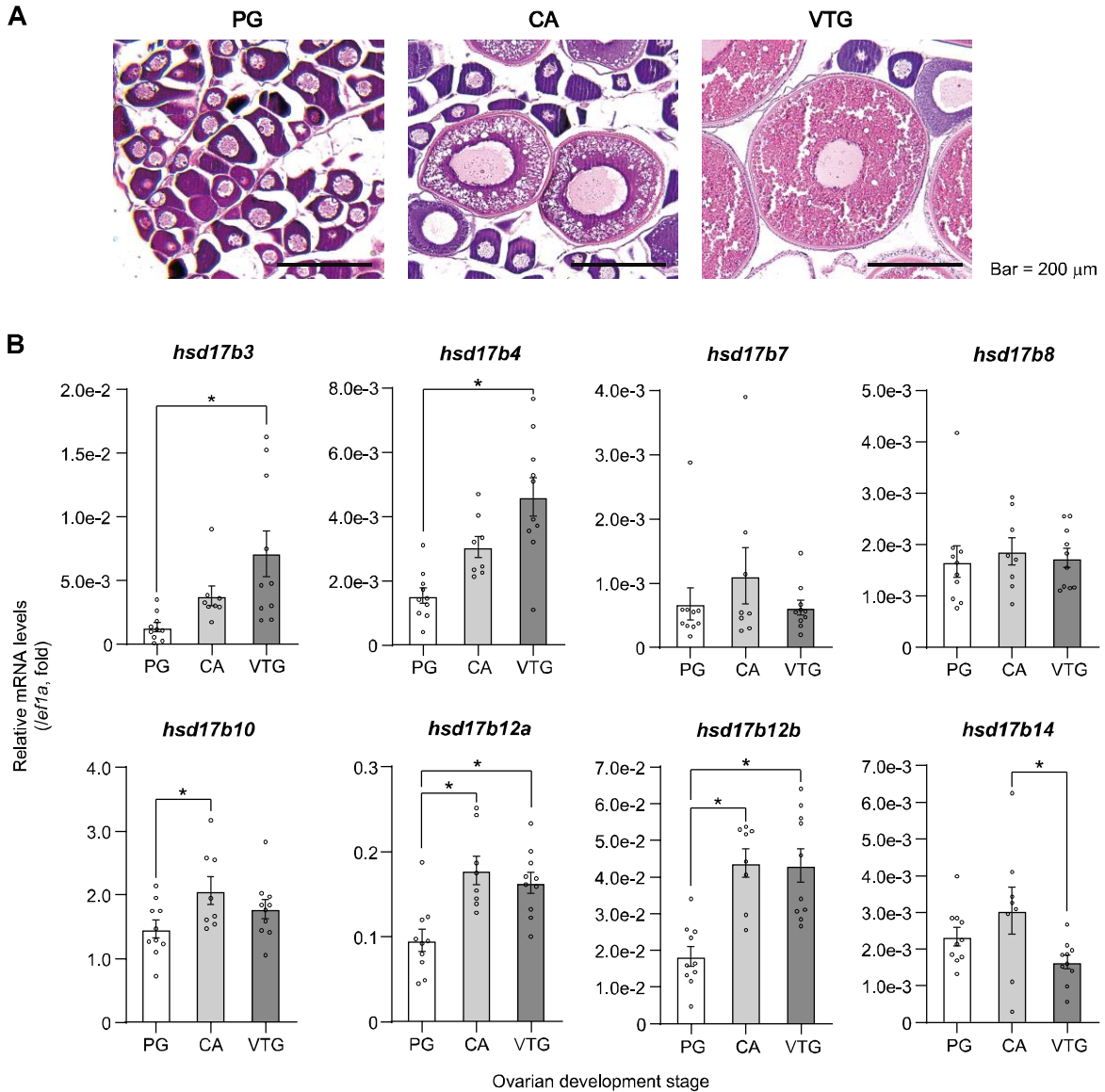


Figure 5

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 $\pm$ SEM (PG and VTG, $n = 10$; CA, $n = 8$). Asterisks indicate significant differences ($p < 0.05$).

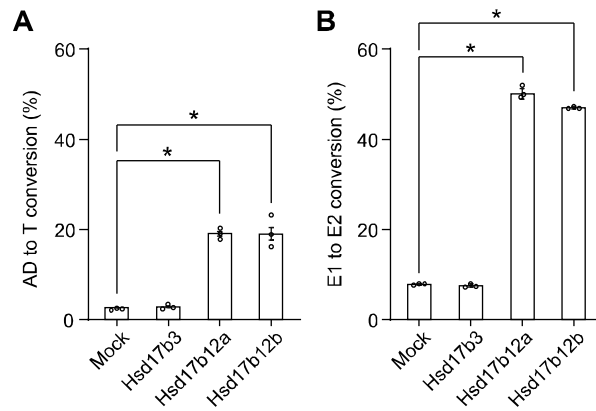


Figure 6

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 $\pm$ SEM of three replicates. Asterisks indicate significant differences ($p < 0.05$) between cells transfected with empty vector (mock) and each Hsd17b expression vector.

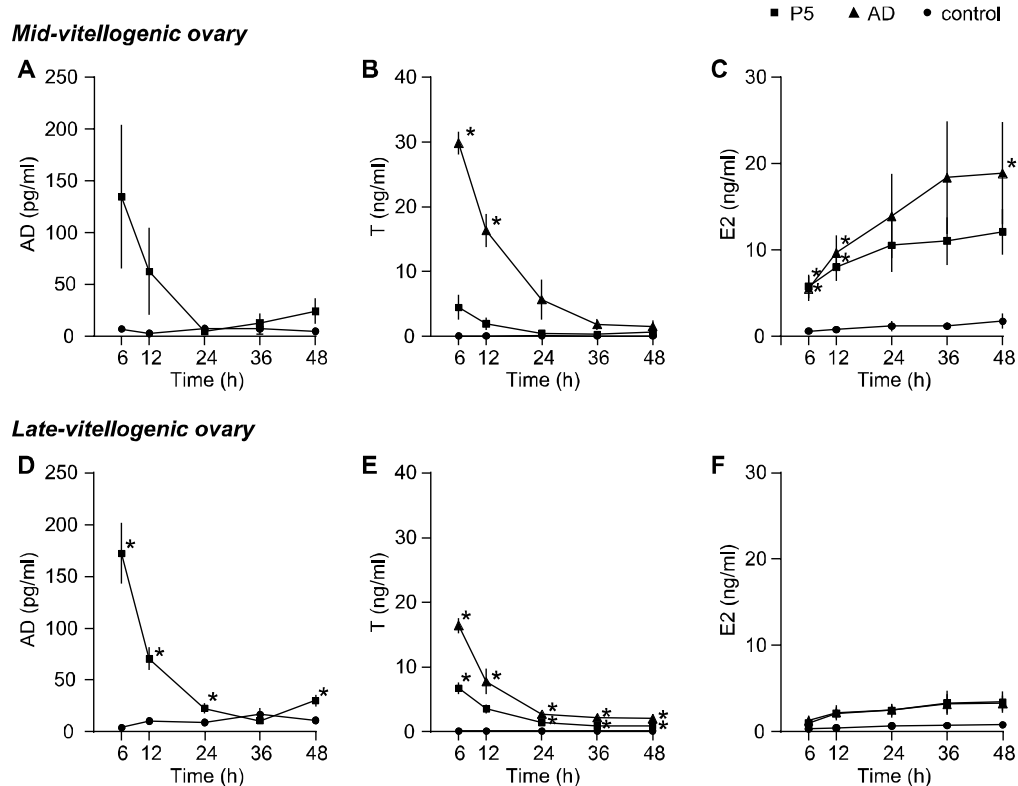


Figure 7

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.