Article

Characterization of Steroid Metabolic Pathways in Established Human and Mouse Cell Models

Therina du Toit [1,2,3,*], Michael Groessl [4], Emanuele Pignatti [1,2], Amanda C. Swart [3,5] and Christa E. Flück [1,2]

[1] Department of BioMedical Research, University of Bern, 3008 Bern, Switzerland
[2] Division of Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics, Bern University Hospital, University of Bern, 3010 Bern, Switzerland
[3] Department of Biochemistry, Stellenbosch University, Stellenbosch 7600, South Africa
[4] Department of Nephrology and Hypertension, Inselspital, Bern University Hospital, University of Bern, 3010 Bern, Switzerland
[5] Department of Chemistry and Polymer Science, Stellenbosch University, Stellenbosch 7600, South Africa.
* Correspondence: therina.dutoit@unibe.ch

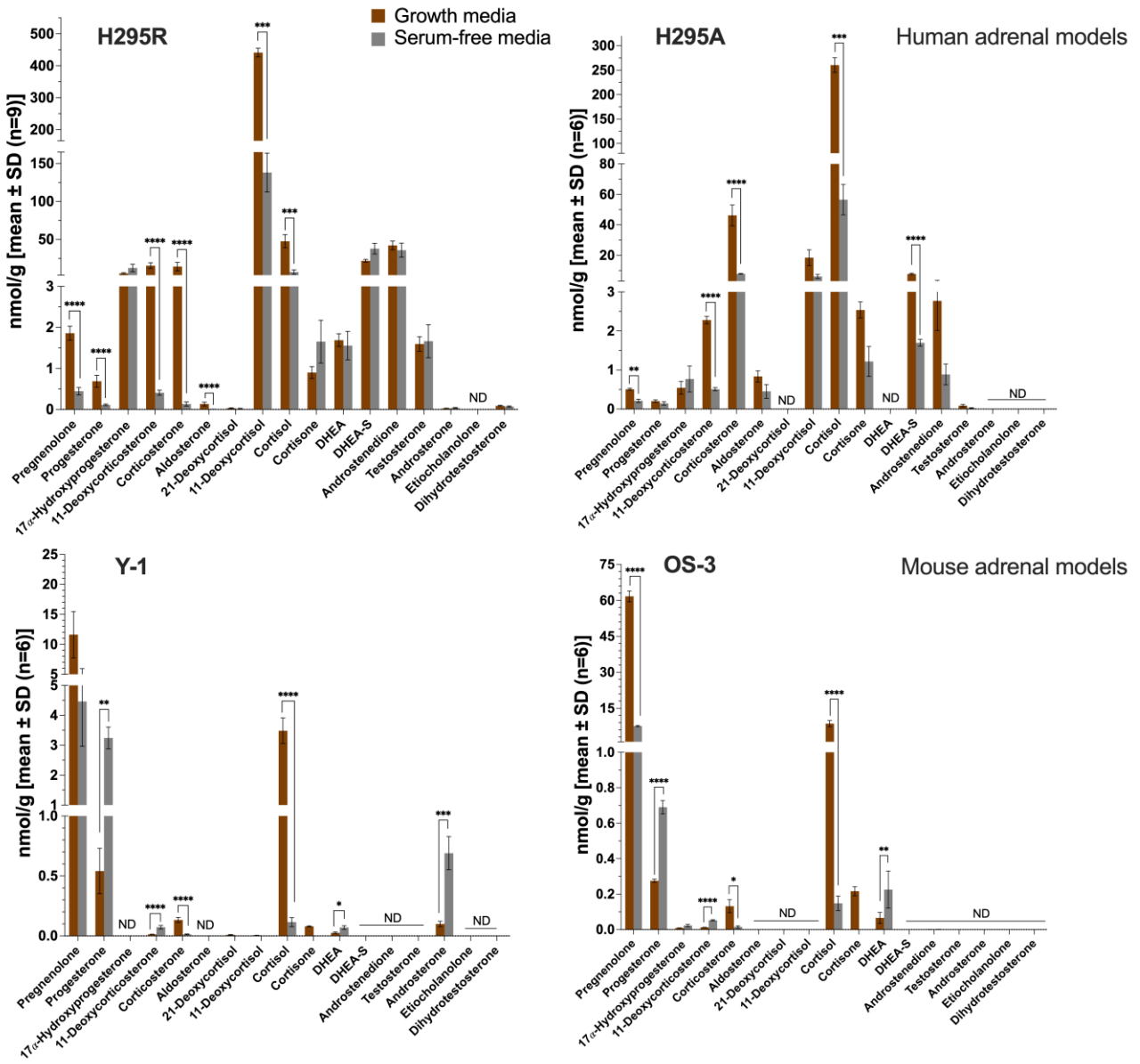


Figure S1. Human and mouse adrenal cell models in complete growth media and serum-free media conditions following a 24 h incubation period. DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; ND, not detected. *p≤0.05, **p≤0.01, ***p≤0.001, **** p<0.0001.

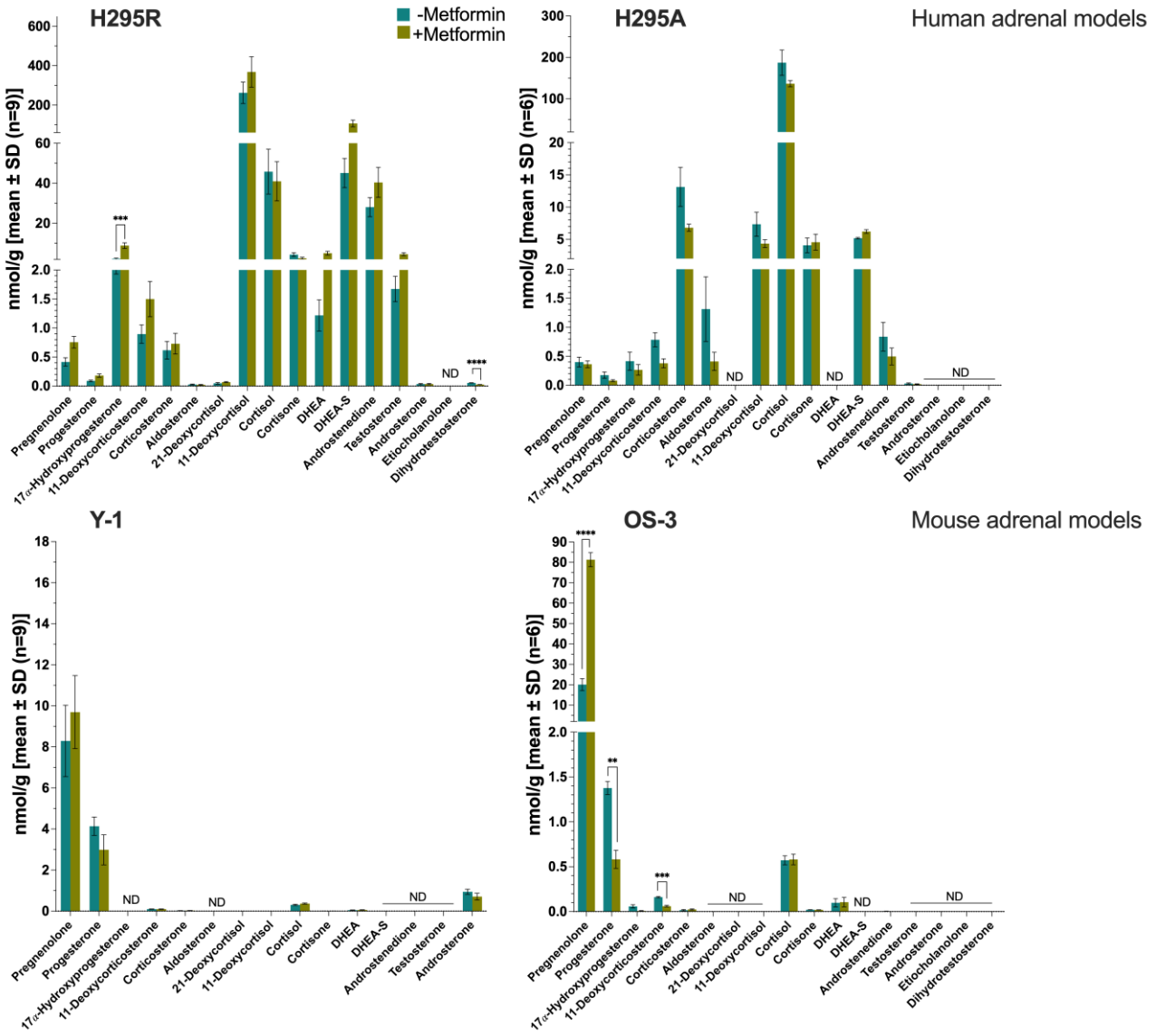


Figure S2. Human and mouse adrenal cell models in serum-free media conditions with and without metformin (10 mM) following a 48 h incubation period. DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; ND, not detected. **p≤0.01, ***p≤0.001, **** p<0.0001.

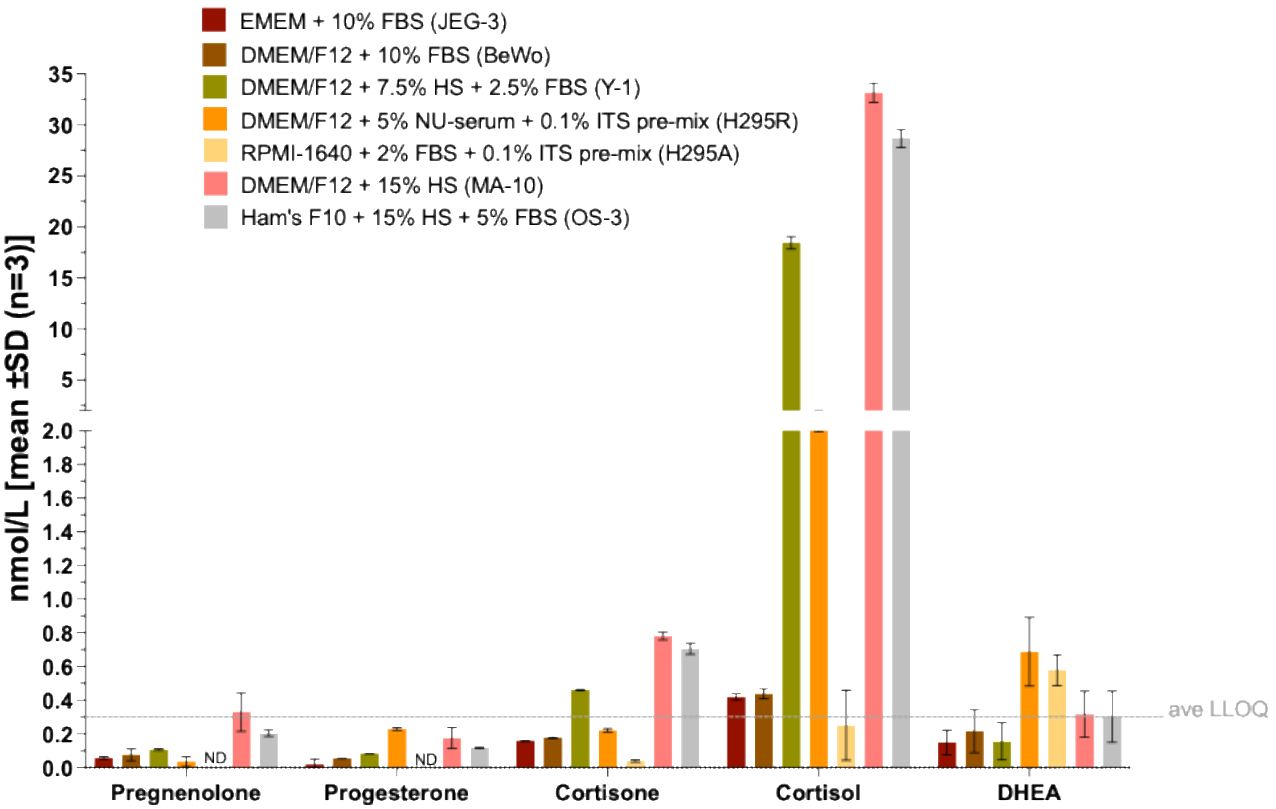


Figure S3. Steroid metabolites quantified in complete growth media (see legend). DHEA, dehydroepiandrosterone; ave. LLOQ, average lower limit of accurate quantification; FBS, fetal bovine serum; HS, horse serum; ITS, insulin-transferrin-selenous acid; ND, not detected.

Table S1. Steroid metabolites measured in the study (LLOQ, lower limit of accurate quantification).

Steroid metabolite	Abbreviation	LLOQ (nmol/L)
Dehydroepiandrosterone	DHEA	0.846
Dehydroepiandrosterone sulfate	DHEA-S	6.252
Androstenedione	-	0.107
Androstenediol	A5	20.00
Testosterone	-	0.105
Dihydrotestosterone	-	0.105
5α-Androstane-3,17-dione	5α-Androstanedione/5αDIONE	6.000
5α-Androstane-3α,17β-diol	5α-Androstanediol/3αDIOL	20.00
11β-Hydroxyandrostenedione	11OH-androstenedione/11OHA4	0.600
11β-Hydroxytestosterone	11OH-testosterone/11OHT	0.150
11-Ketoandrostenedione	11K-androstenedione/11KA4	0.600
11-Ketotestosterone	11K-testosterone/11KT	0.600
11β-Hydroxy-5α-androstane-3,17-dione	11OH-5α-androstanedione/11OH5αDIONE	2.000
11β-Hydroxyandrosterone	11OH-androsterone/11OHAn	2.000
11-Ketoandrosterone	11K-androsterone/11KAn	2.000
Androsterone	-	0.420
Etiocholanolone	-	0.210
Pregnenolone	-	0.771
17α-Hydroxypregnenolone	17OH-pregnenolone/17OHP5	20.00
Progesterone	-	0.476
16α-Hydroxyprogesterone	16OH-progesterone/16OHP4	0.600
6α-Hydroxyprogesterone	6αOH-progesterone/6αOHP4	0.600
6β-Hydroxyprogesterone	6βOH-progesterone/6βOHP4	1.000
11α-Hydroxyprogesterone	11αOH-progesterone/11αOHP4	1.500
11β-Hydroxyprogesterone	11βOH-progesterone/11βOHP4	0.100
11-Ketoprogesterone	11K-progesterone/11KP4	0.200
5α-Pregnanetrione/11-Ketodihydroprogesterone	11K-dihydroprogesterone/11KDHP4	1.000
17α-Hydroxyprogesterone	17OH-progesterone	0.092
21-Deoxycortisol	-	0.088
17α20α-Dihydroxyprogesterone	17,20-diOH-progesterone/17,20diOHP4	0.600
17α-Hydroxypregnanolone (5β-pregnane-3β,17α-diol-20-one)	17OHTHP	10.00
Pregnanetriol	Ptriol	15.00
Pregnanetriolone/11-Ketopregnanetriol	11KPtriol	6.000
20α-Hydroxyprogesterone	20αOH-progesterone/20αOHP4	0.600
20β-Hydroxyprogesterone	20βOH-progesterone/20βOHP4	0.600
5α/β-Dihydroprogesterone	5α/βDHP4	6.000
Pregnanolone (3α,5β-THP)	THP	15.00
5α-Pregnanolone (3α,5α-THP; allopregnanolone)	5αTHP	10.00
5α/β-Pregnan-3β-ol-20-one (3β-hydroxy-5α-THP)	3βTHP	15.00
5α/β-Pregnane-3α, 20α-diol	20OHTHP	100.0
6α-Hydroxypregnanolone	6OHTHP	10.00
5α/β-Pregnane-3β, 20α-diol	20OH-3βTHP	20.00
11-Deoxycorticosterone	-	0.092

Corticosterone	-	0.705
Aldosterone	-	0.085
11-Deoxycortisol	-	0.088
Cortisol	-	0.378
Cortisone	-	0.177

Table S2. Steroidogenic enzyme mRNA expression quantified in cell models based on findings in literature.

Cell model	**mRNA expression**	**References**
H295R	*STAR, CYP11A1, HSD3B2, CYP17A1, CYB5, CYP21A2, POR, CYP11B1, CYP11B2, SULT2A1, HSD17B5, HSD17B3*	(1-6)
H295A	*STAR, CYP11A1, HSD3B2, CYP17A1, CYB5, CYP21A2, POR, CYP11B1, SULT2A1, HSD17B5, HSD17B3*	(2, 7)
Y-1	*Star, Cyp11a, Cyp11b1*	(1, 8)
OS-3	-	
BeWo	*CYP11A1, CYP17A1, 11βHSD2, AKR1C3, CYP19A1, SRD5A1, HSD17B1, CYP3A4*	(9-11)
JEG-3	*CYP11A1, HSD3B1, CYP17A1, AKR1C3, CYP19A1, HSD11B1, SRD5A1, HSD17B1, HSD11B2, CYP3A4, HSD17B7, HSD17B12, STS*	(9-13)
MA-10	*Star, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b1, Hsd17b3, Akr1c14, Srd5a1*	(14, 15)

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