

## Supplementary Tables and Figures

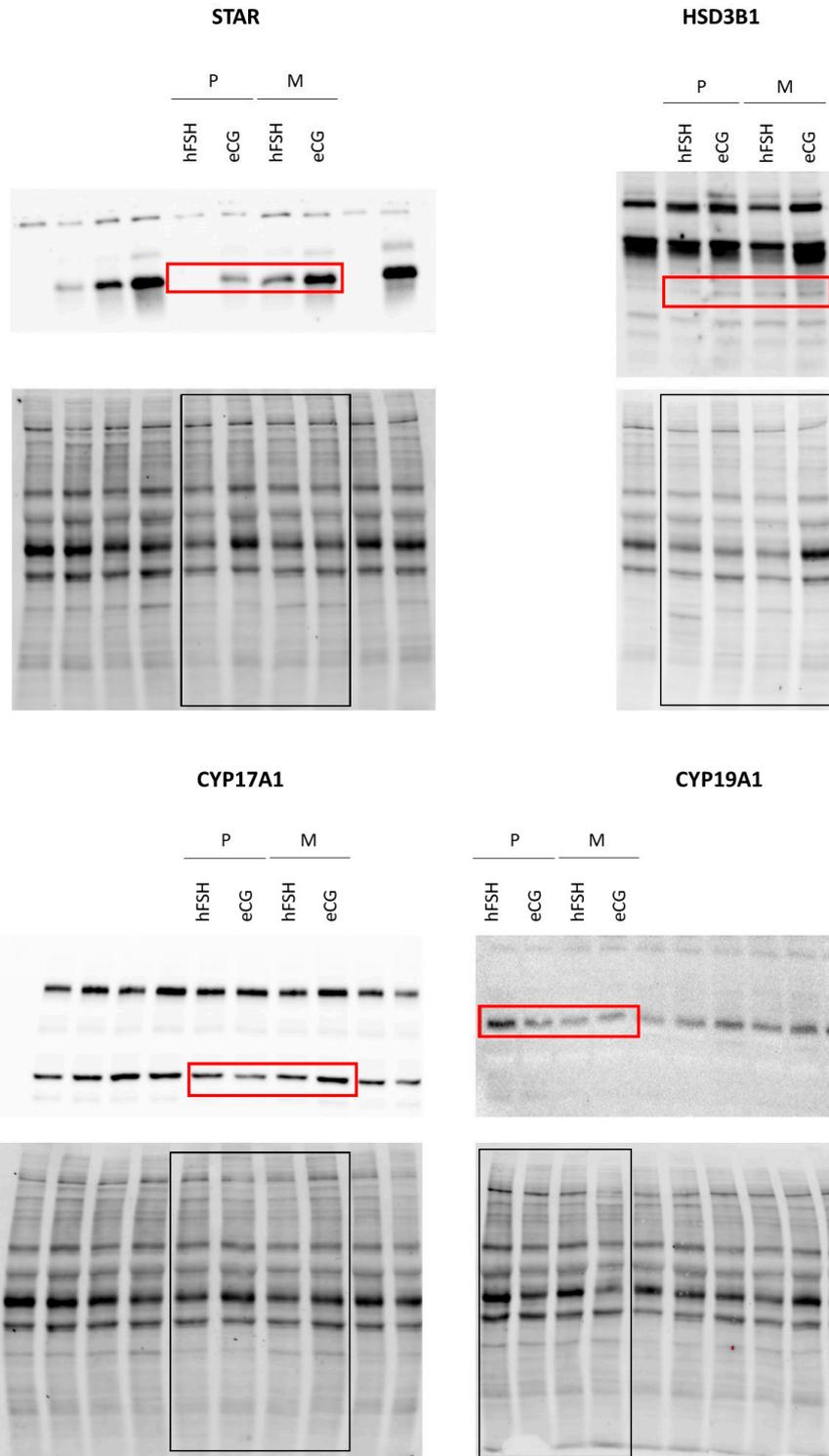
Supplementary Table S1. List of antibodies used in study.

Peptide/Protein Target	Name of Antibody	Catalog No., Name of Source	Species Raised in Monoclonal or Polyclonal	Dilution used
<b>Prostaglandin assays</b>				
PGE <sub>2</sub>	Anti-Prostaglandin E <sub>2</sub> Antibody	P5164 Sigma-Aldrich	Rabbit, polyclonal	1:200
PGFM	Anti-PGFM serum	WS4468-7 donated by Dr William Silvia	Rabbit, polyclonal	1:10000
<b>Western blot</b>				
CYP17A1	Anti- CYP17A1 Antibody	ab125022 Abcam	Rabbit, monoclonal	1:1000
CYP19A1	Anti-CYP19A1 Antibody	MCA2077S Bio-Rad	Mouse, monoclonal	1:250
FSHR	Anti-FSHR Antibody	bs 0895R Biossua	Rabbit, polyclonal	1:400
HSD3B1	Anti- HSD3B1 Antibody	ab55268 Abcam	Mouse, monoclonal	1:100
LHCGR	Anti- LHCGR Antibody	AD2716317 donated by Dr Marco Banoni	Mouse, monoclonal	1:600
PTGFS	Anti- PTGFS Antibody	AV48180 Sigma	Rabbit, polyclonal	1:200
STAR	Anti-StAR Antibody	ab96637 Abcam	Rabbit, polyclonal	1:400
GAPDH	Anti- GAPDH Antibody	MA5-15738 Thermo-Fisher Scientific	Mouse, monoclonal	1:2000
Anti-rabbit, secondary antibodies	Immun-Star Goat Anti- Rabbit (GAR)-HRP Conjugate	1705046 Bio-Rad	Goat, polyclonal	1:20000
Anti-mouse, secondary antibodies	Immun-Star Goat Anti- Mouse (GAM)-HRP Conjugate	1705047 Bio-Rad	Goat, polyclonal	1:20000

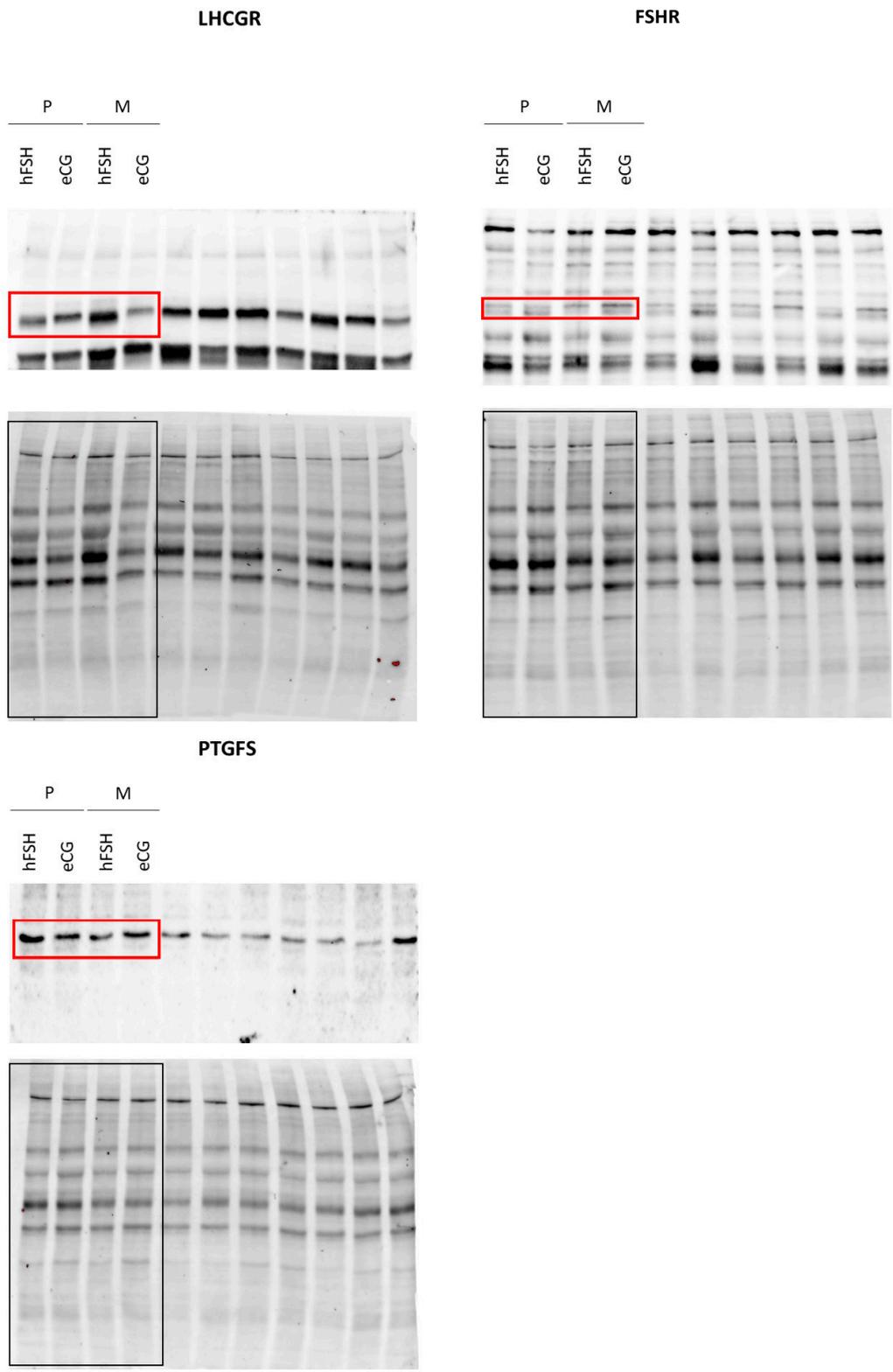
Supplementary Table S2. List of genes assayed in real-time PCR.

<b>Gene symbol</b>	<b>Gene name</b>	<b>Accession number</b>	<b>TaqMan Assays ID</b>	<b>Product length (bp)</b>
<i>STAR</i>	Steroidogenic Acute Regulatory Protein	NM_213755.2	Ss03381250_u1	73
<i>HSD3B1</i>	Hydroxy-delta-5-steroid Dehydrogenase, 3 Beta- and Steroid Delta-isomerase 1	NM_001004049.2	Ss03391752_m1	72
<i>CYP17A1</i>	Cytochrome P450 Family 17 Subfamily A Member 1	NM_214428.1	Ss03394945_m1	61
<i>CYP19A1</i>	Cytochrome P450 Family 19 Subfamily A Member 1	NM_214429.1	Ss03384876_u1	72
<i>PTGES</i>	Prostaglandin E Synthase	NM_001038631.1	Ss03392129_m1	70
<i>LHCGR</i>	Luteinizing Hormone/Choriogonadotropin Receptor	NM_214449.1	Ss03384991_u1	64
<i>FSHR</i>	Follicle Stimulating Hormone Receptor	NM_214386.3	Ss03384581_u1	99
<i>ACTB*</i>	Beta-Actin	XM_003357928.4	Ss03376081_u1	77
<i>GAPDH*</i>	Glyceraldehyde 3-phosphate Dehydrogenase	NM_001206359.1	Ss03375435_u1	75
<i>HPRT1*</i>	Hypoxanthine-guanine Phosphoribosyltransferase	NM_001032376.2	Ss03388274_m1	73

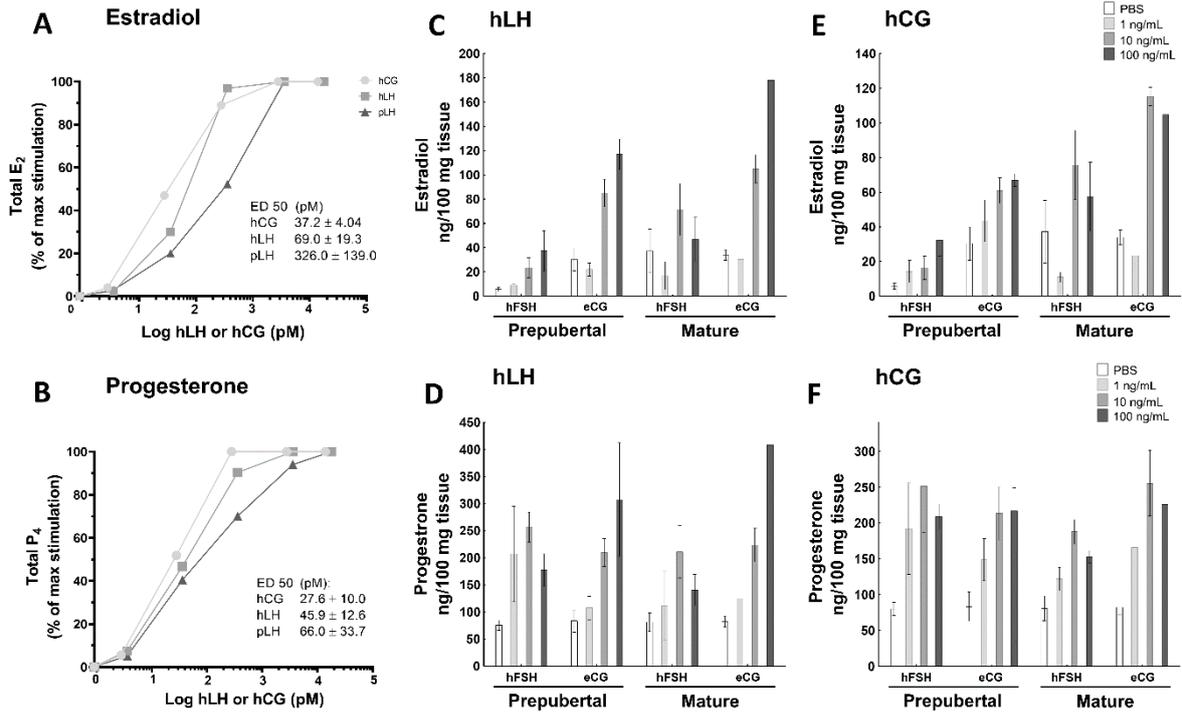
\*Reference genes



**Supplementary Figure S1. Uncropped blots for STAR, HSD3B1, CYP17A1, and CYP19A1 proteins in follicles collected from prepubertal and mature gilts challenged with hFSH or eCG.** In each upper panel, full blot showing STAR, HSD3B1, CYP17A1, and CYP19A1 protein expression are shown. Lower panels represent equivalent TGX Stain-Free gel showing total protein. Red boxes indicate areas presented in Figure 3. P – prepubertal, M – mature.



**Supplementary Figure S2. Uncropped blots for LHCGR, FSHR, and PTGFS proteins in follicles collected from prepubertal and mature gilts challenged with hFSH or eCG.** In each upper panel, full blot showing LHCGR, FSHR, and PTGFS protein expression are showed. Lower panels represent equivalent TGX Stain-Free gel showing total protein. Red boxes indicate areas presented in Figure 4. P – prepubertal, M – mature.



**Supplementary Figure S3. Dose-response experiment.** Stimulation of total E<sub>2</sub> (A) and P<sub>4</sub> (B) production by hLH and hCG. Preovulatory follicles were stimulated by increasing doses of hCG and LH. E<sub>2</sub> and P<sub>4</sub> levels were normalized as percentage of the maximal response. All the results are represented as Means ± SEM in a logarithmic X-axis, then non-linear regressions were plotted. Concentrations of E<sub>2</sub> (C, E) and P<sub>4</sub> (D, F) release in preovulatory follicles walls explants exposed to r-hLH or r-hCG (1 ng/mL, 10 ng/mL and 100 ng/mL) collected from prepubertal and mature gilts challenged with hFSH or eCG.