

Supplementary Materials:

Supplementary Table S1: List of qRT-PCR primers.

Gene Name	Forward	Reverse	Accession #
GAPDH	TCAACGGCACAGTCAAGG	ACATACTCAGCACCAGCATCAC	NM_001034034
GSK3 β	AGTATACACCAACTGCCCGACT	TGAAGTTGAAGAGTGCAGGTGT	NM_001101310.1
β -catenin	AATCCGAGCTGGACCTCAATGACA	TATCATGTCCAAGCAGCCCGAGAA	NM_174637.4
Axin2	TGCTTATCTCTTCCGGACTTTC	CGCTTTGGCTACTCGTAAAGTT	XM_024979963.1
c-Myc	TTTTCTCCGTCCTCTGACTCTC	TTCTCATCCTCTTGTTCTTCC	NM_001046074.2
CyclinD1	TGAAGGAGACCATTCCCTTG	CCACTTGAGCTTGTTACCA	NM_007631.2
PPAR α	ATATTTCCCTCTTTGTGGCTGC	ATGGTTGTTCTGTAGGTGGAGT	XM_024991367
PPAR γ	GACTTCTCCAGCATTCCACTC	ATACAGGCTCCACTTTGATTGC	NM_181024
PPAR δ	AGAGCACTCACTTCCTTCCAG	GTTGCGGTTCTTCTTCTGGATT	XM_024983411
Cpt1a	GAGGGAGACTTTACACGGATGA	AGATGTATTCTCCACCAGTC	NM_001304989
Pdk4	GGTGATTGTTGTCTTGGGGAAA	AATTATCCATCACAGGCGTTGG	NM_001101883

Supplementary Table S2: List of antibodies used:

Target	Cat #	Manufacturer
β -catenin	ab32572	Abcam
Oct4	Sc-5279	Santa Cruz
Cdx2	AM3920119	Bio Genex
c-Myc	D84C12	Cell Signaling
Cox2	Sc-19999	Santa Cruz
PPAR δ	LS-C437498	LS-BioScience
Cpt1a	Sc-393070	Santa Cruz
DAPI	62248	Thermo Fisher scientific
FITC	B2119	Santa Cruz
TRITC	A6071	Invitrogen

Supplementary Table S3: Effect of PPAR δ inhibitor on bovine blastocyst development and hatching:

Groups	Oocytes, n	Speculated Zygote, n	Cleaved Embryos, n %	Total Blastocyst %	Hatched Blastocyst %
Control	391	369	288(78.0 \pm 0.9) ^b	103(28.0 \pm 0.3) ^c	45(44.4 \pm 3.1) ^d
(8 μ M)	340	328	253(77.1 \pm 0.4) ^b	88(27.0 \pm 0.5) ^{b,c}	33(37.1 \pm 0.8) ^c
(10 μ M)	337	331	246(74.1 \pm 0.6) ^a	82(25.0 \pm 0.5) ^b	27(32.6 \pm 1.9) ^c
(12 μ M)	338	317	236(74.0 \pm 0.5) ^a	57(18.3 \pm 0.8) ^a	12(20.9 \pm 2.2) ^b
(15 μ M)	208	199	148(74.6 \pm 1.3) ^a	32(16.2 \pm 1.7) ^a	3(9.4 \pm 4.0) ^a

^{a,b} $p < 0.05$ with different superscript in the column indicate significant difference.

Supplementary Figure S1:

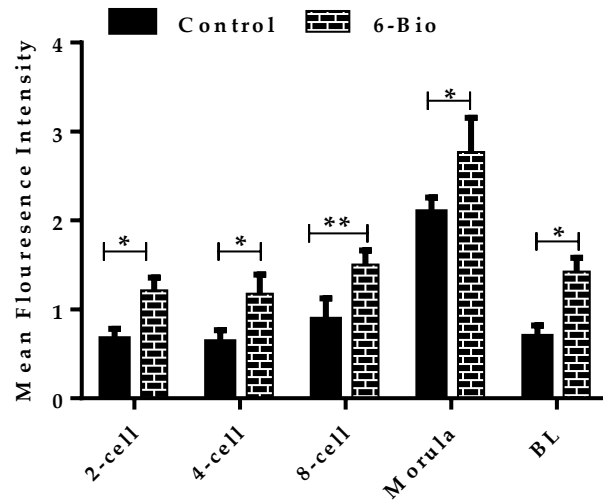


Figure S1. Mean fluorescence intensity of β -catenin in control and 6-Bio treated groups at different stages of embryonic development. Bar graph data of mean fluorescence intensities presented as a means \pm SEM from three individual sets of experiment, including n = 20 BLs per group in each replicate. * p < 0.05; ** p < 0.01; indicates significant difference.

Supplementary Figure S2:

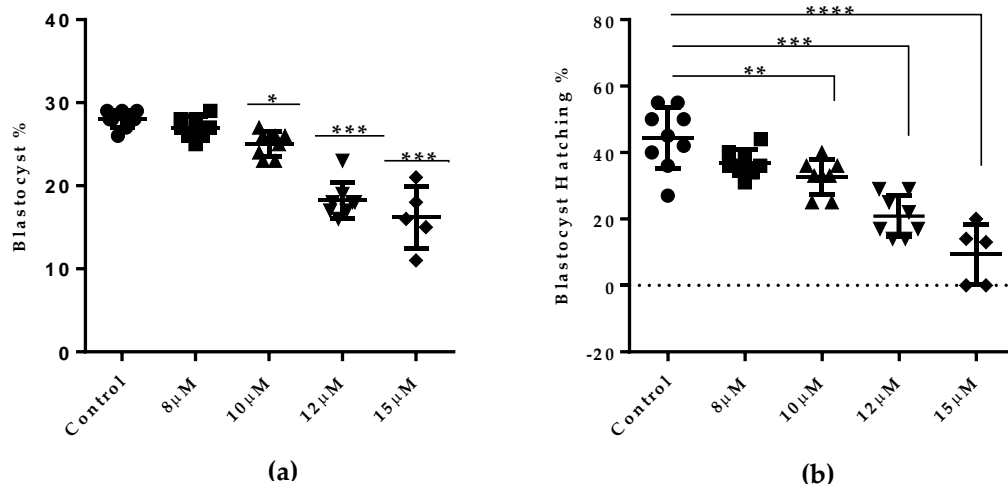
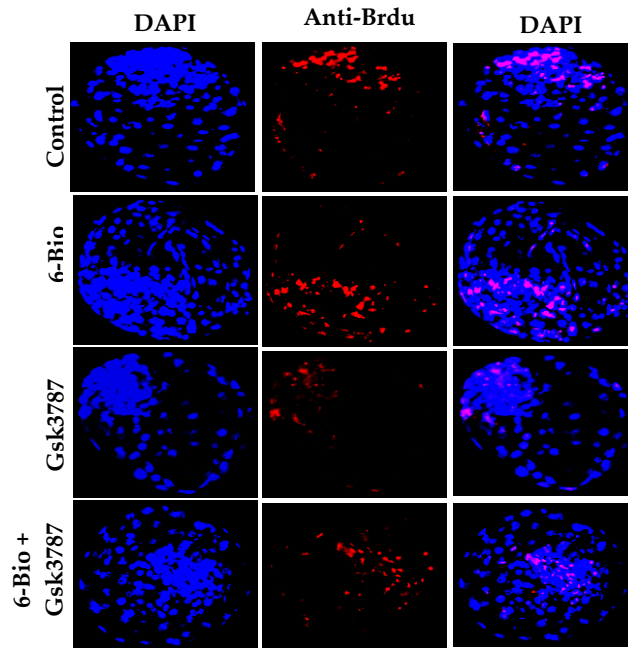
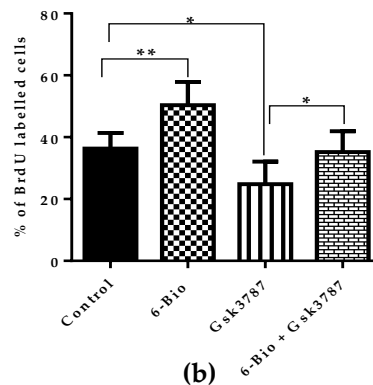


Figure S2: Effect of dose dependent inhibition of PPAR function on BL development and hatching rate. (a-b) Graphical data shows the % of bovine blastocyst development and hatching during different concentration of Gsk3787. Data presented as a means \pm SEM from three individual sets of experiment, including n = 20 BLs per group in each replicate. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.000; indicates significant difference.

Supplementary Figure S3:



(a)



(b)

Figure S3: BrdU proliferation assay in Wnt agonist and PPAR δ antagonist treated group. (a) Immunofluorescence staining showing the anti-BrdU labelled cells in different treatment groups. **(b)** Graphical data shows the % of BrdU labelled cells in different treatment groups. Data presented as means \pm SEM from three individual sets of experiment, including n = 20 BLs per group in each replicate. * p < 0.05; ** p < 0.01; indicates significant difference.

Supplementary Figure S4:

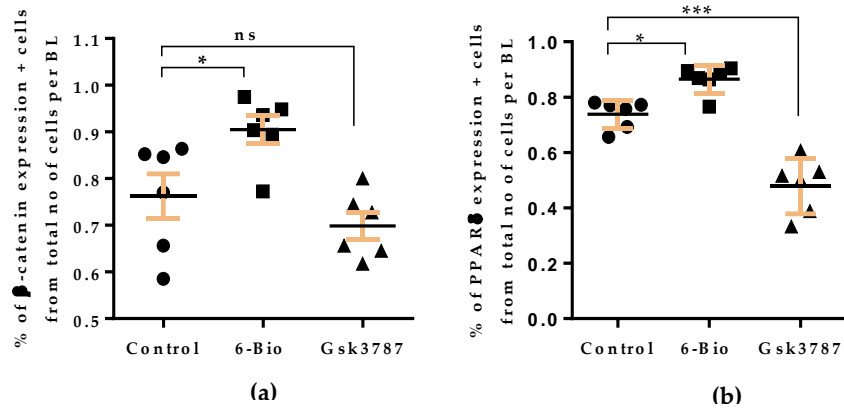


Figure S4: Quantitative analysis of β -catenin and PPAR δ expression. (a-b) graphical data represents the percentage of β -catenin and PPAR δ expression positive cells from total number of cells per BL in each group. \pm SEM from three individual sets of experiments (n = 20 BLs). ns, non-significant, * p < 0.05; *** p < 0.001 indicates significant difference.