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Supplementary Material

Iloprost supports early development of *in vitro*-produced porcine embryos through activation of the phosphatidylinositol 3-kinase/AKT signalling pathway

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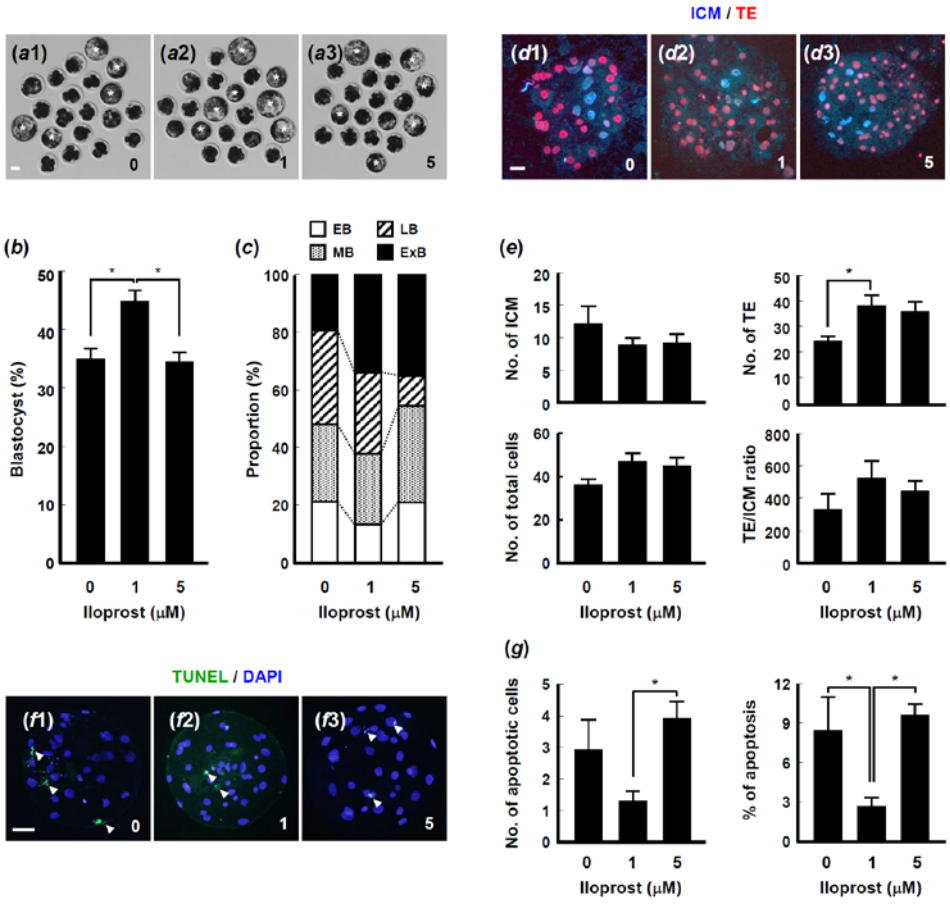

Fig. S1. Effects of iloprost on the developmental competence of porcine IVF embryos. Representative photographs of the IVF blastocysts (white asterisks) that developed at the indicated doses of iloprost (*a*1–*a*3). Bar = 50 μm. Quantification of the blastocyst development rate in the indicated groups (*b*). The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$). Quantification of the proportion of each blastocyst stage in the indicated groups (*c*). Differential staining of ICM and TE using blastocysts cultured under the indicated treatment conditions (*d*1–*d*3). Merged images between Hoechst 33342 (blue; ICM) and PI (red; TE) signals are shown. Bar = 50 μm. Quantification of the total, ICM, TE cell numbers, and TE/ICM ratios in the indicated groups (*e*). The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$). Apoptosis detection analysis in blastocysts from the indicated groups. Merged images (light green) between TUNEL (green, white arrow) and DAPI (blue) signals are shown (*f*1–*f*3). Bar = 50 μm. Quantification of the number (*g*, left) and proportion (*g*, right) of apoptotic cells in the indicated groups. The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$).

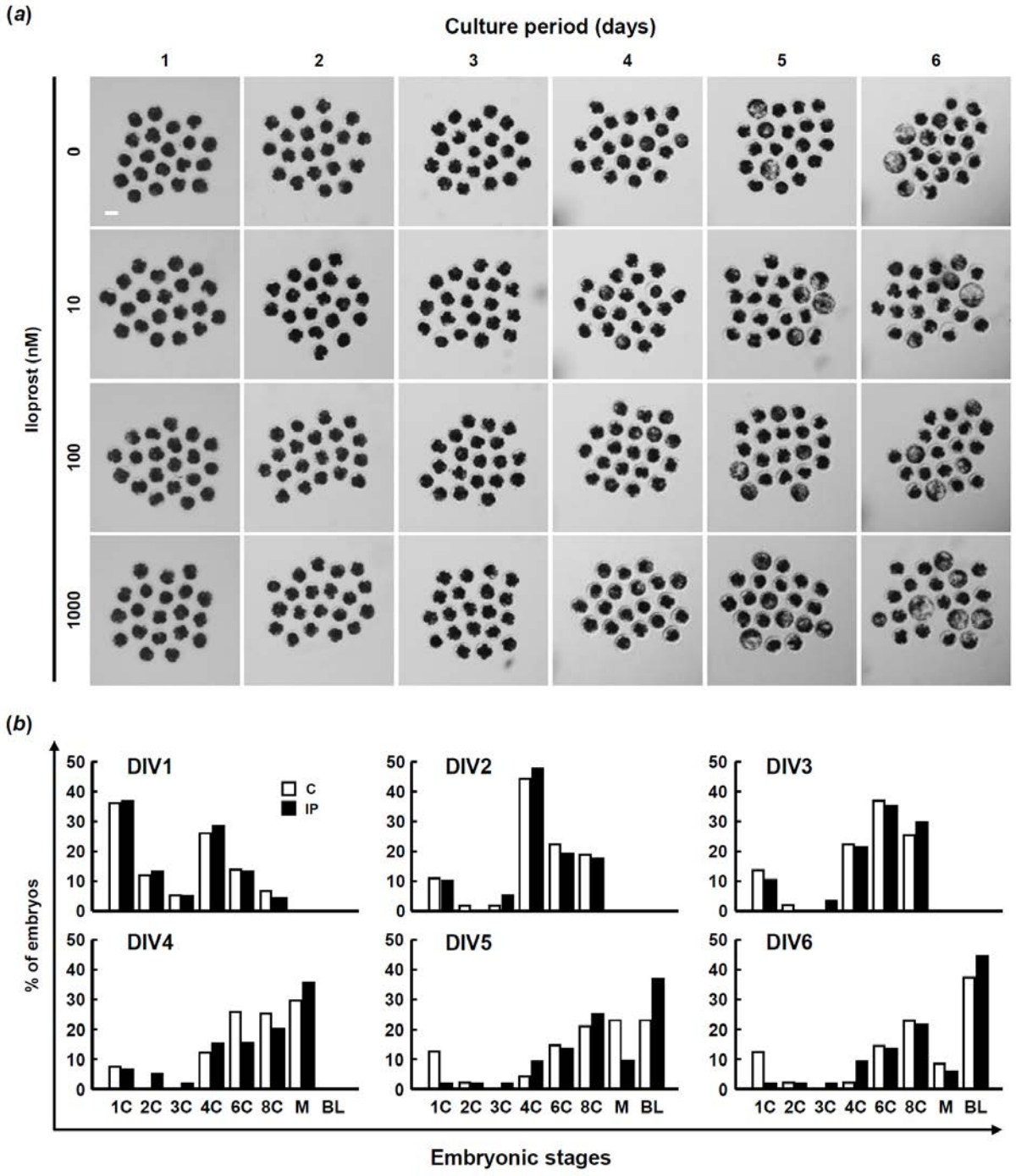

Fig. S2. Developmental kinetics of porcine IVF embryos for *in vitro* culture. Representative photographs of the IVF blastocysts (white asterisks) that developed at the indicated doses of iloprost (*a*). Bar = 50 μm. Quantification of the blastocyst development rate in the indicated groups (*b*).

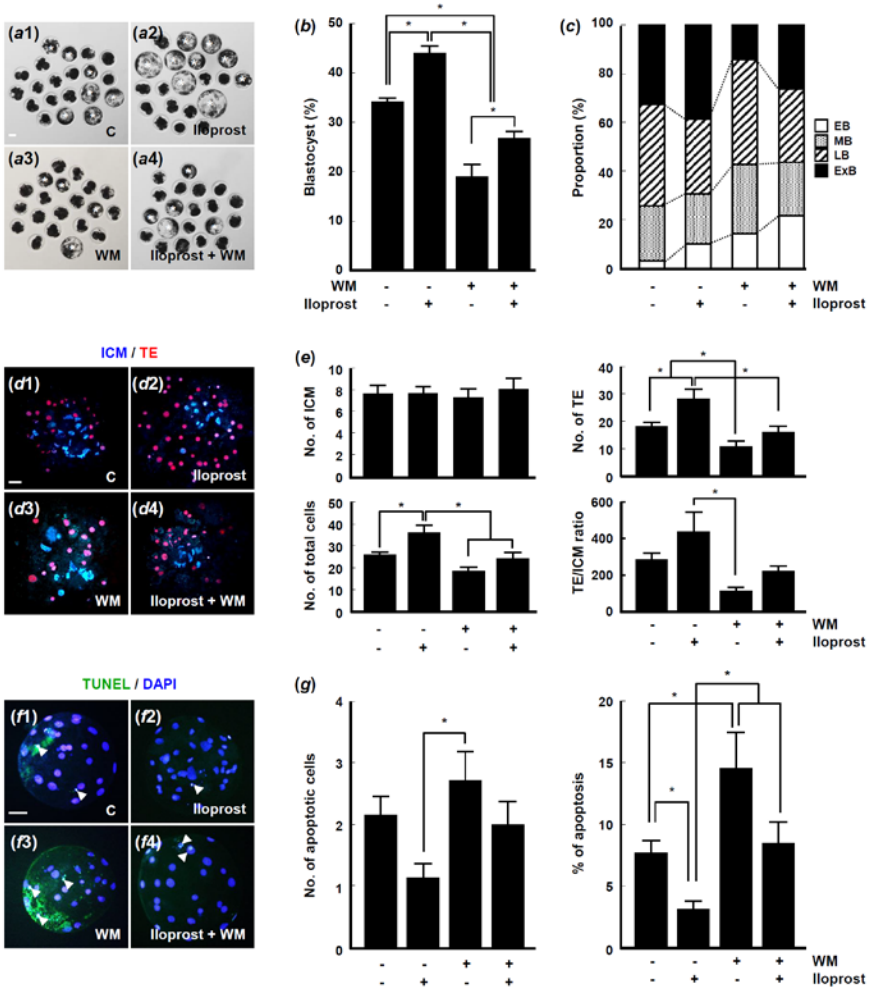

Fig. S3. Effects of combined treatment of iloprost and Wortmannin on the developmental competence of porcine PA embryos. Representative photographs of the PA blastocysts (white asterisks) that developed at the indicated doses of iloprost (*a*1–*a*4). Bar = 50 μm. Quantification of the blastocyst development rate in the indicated groups (*b*). The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$). Quantification of the proportion of each blastocyst stage in the indicated groups (*c*). Differential staining of ICM and TE using blastocysts cultured under the indicated treatment conditions (*d*1–*d*4). Merged images between Hoechst 33342 (blue; ICM) and PI (red; TE) signals are shown. Bar = 50 μm. Quantification of the total, ICM, TE cell numbers, and TE/ICM ratios in the indicated groups (*e*). The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$). Apoptosis detection analysis in blastocysts from the indicated groups. Merged images (light green) between TUNEL (green, white arrow) and DAPI (blue) signals are shown (*f*1–*f*4). Bar = 50 μm. Quantification of the number (*g*, left) and proportion (*g*, right) of apoptotic cells in the indicated groups. The data are from three independent experiments, and values represent the means ± s.e.m. ($^{*}P < 0.05$).

Table S1. Effect of iloprost doses on post-blastulation development of porcine IVF blastocysts

Data are the mean ± s.e.m. EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; ExB, expanded blastocysts

Iloprost (nM)	No. of blastocysts examined	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	ExB
0	16	21.0 ± 4.2	32.4 ± 3.8	30.5 ± 6.7	16.2 ± 8.5
10	16	16.7 ± 8.3	34.7 ± 16.4	26.4 ± 6.0	22.2 ± 2.8
100	18	10.7 ± 7.4	15.1 ± 8.3	37.9 ± 2.8	36.3 ± 8.3
1000	27	9.2 ± 1.9	21.1 ± 4.4	22.2 ± 2.1	47.5 ± 1.3

Table S2. Effect of iloprost doses on post-blastulation development of porcine IVF blastocysts

Data are the mean ± s.e.m. Values with different superscript letter within a column differ significantly ($P < 0.05$). EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; ExB, expanded blastocysts

Iloprost (μM)	No. of blastocysts examined	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	ExB
0	52	20.9 ± 1.5^{a}	27.3 ± 2.2	32.6 ± 0.7^{a}	19.3 ± 0.4^{a}
1	53	13.1 ± 0.3^{b}	24.7 ± 3.4	28.5 ± 2.5^{a}	33.7 ± 1.5^{b}
5	57	20.7 ± 2.7^{ab}	33.9 ± 2.4	10.4 ± 2.7^{b}	34.9 ± 1.7^{b}

Table S3. Effect of Wortmannin doses on post-blastulation development of porcine IVF blastocysts

Data are the mean ± s.e.m. Values with different superscript letters within a column differ significantly ($P < 0.05$). EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; ExB, expanded blastocysts

Wortmannin (μM)	No. of blastocysts examined	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	ExB
0	46	30.2 ± 1.6[a]	27.5 ± 9.2	21.7 ± 4.1[a]	20.6 ± 6.3[a]
1	26	53.3 ± 3.3[ab]	31.7 ± 9.3	15.0 ± 7.6[ab]	0.0 ± 0.0[b]
5	20	61.1 ± 5.6[ab]	38.9 ± 5.6	0.0 ± 0.0[b]	0.0 ± 0.0[b]
10	10	83.3 ± 16.7[b]	16.7 ± 16.7	0.0 ± 0.0[b]	0.0 ± 0.0[b]

Table S4. Effect of combined treatment of iloprost and Wortmannin on post-blastulation development of porcine IVF blastocysts

Data are the mean ± s.e.m. Values with different superscript letters within a column differ significantly ($P < 0.05$). EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; ExB, expanded blastocysts; WM, Wortmannin

Groups	No. of blastocysts examined	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	ExB
Control	26	10.7 ± 3.6	32.9 ± 9.6	25.0 ± 9.0	31.5 ± 2.9[a]
Iloprost	36	5.4 ± 3.2	17.2 ± 3.8	29.9 ± 5.9	47.5 ± 5.3[a]
WM	12	18.8 ± 12.0	29.2 ± 10.5	52.1 ± 9.9	0.0 ± 0.0[b]
Iloprost+WM	19	10.0 ± 10.0	17.2 ± 10.2	37.8 ± 15.4	35.1 ± 10.7[a]

Table S5. Effect of combined treatment with iloprost and Wortmannin on post-blastulation development of porcine PA blastocysts

Data are the mean ± s.e.m. EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; ExB, expanded blastocysts; WM, Wortmannin

Groups	l	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	ExB
Control	31	3.6 ± 3.6	25.9 ± 6.0	39.9 ± 4.1	30.7 ± 5.8
Iloprost	39	11.1 ± 4.5	20.8 ± 1.4	30.5 ± 2.8	37.5 ± 4.2
WM	14	14.6 ± 8.6	29.2 ± 2.4	41.7 ± 4.8	14.6 ± 8.6
Iloprost+WM	23	18.8 ± 6.6	28.8 ± 10.9	27.5 ± 4.3	25.0 ± 2.0

Table S6. Effect of combined treatment with iloprost and Wortmannin on post-blastulation development of porcine SCNT blastocysts

Data are the mean ± s.e.,. Values with different superscript letters within a column differ significantly ($P < 0.05$). EB, indicates early blastocysts; MB, mid-blastocysts; LB, late blastocysts; HB, hatched blastocysts; WM, Wortmannin

Groups	No. of blastocysts examined	Proportion of blastocysts developed to the following stages (%)			
		EB	MB	LB	HB
Control	31	10.2 ± 6.5	21.5 ± 6.5 ^{ab}	29.1 ± 5.5	39.2 ± 3.2 ^{ab}
Iloprost	42	7.2 ± 3.7	19.1 ± 4.1^{a}	23.2 ± 4.1	50.5 ± 3.4^{a}
WM	29	11.8 ± 6.0	47.9 ± 4.1^{b}	26.2 ± 5.5	14.0 ± 2.1^{b}
Iloprost+WM	39	25.0 ± 14.4	28.3 ± 9.0 ^{ab}	24.7 ± 10.6	22.0 ± 11.3^{b}