Fig.S1. Effects of different concentrations of AICAR, Met and Compound C (C) on total motility (A-C), progressive motility (D-F) and acrosome reaction (G-I). Bars represent mean values ± SEM (n = 5 independent replicates). Different lower-case letters denote significant differences (p < 0.05).
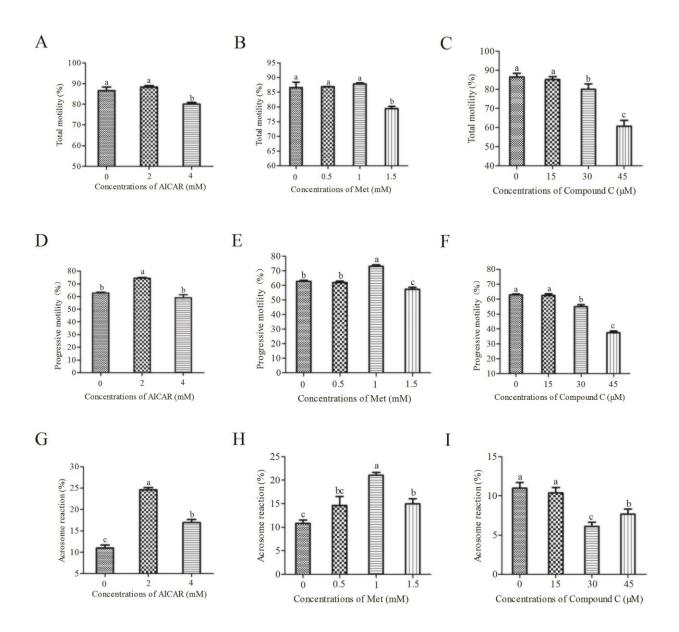

Fig.S2. (A) Status of the goat sperm acrosome stained with FITC-PNA/PI. The white arrow indicates the sperm without acrosome reaction exhibiting green fluorescence in acrosome and red fluorescence in nuclei, while the blue arrow indicates the sperm with acrosome reaction exhibiting

red fluorescence only in nuclei. (B) Status of the goat sperm membrane integrity stained with

SYBR-14/PI. White arrow indicates the sperm with membrane integrity (stained with SYBR-14),

black arrow indicates the sperm with slightly damaged membrane (stained with SYBR-14 and PI),

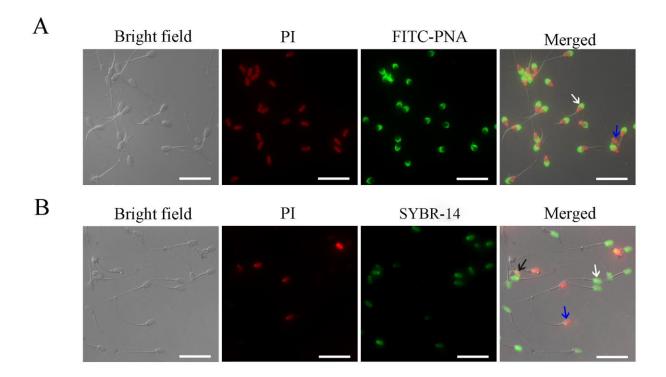blue arrow indicates the sperm with damaged membrane (stained with PI). Bars = 30 μm.

Fig. S3. Densitometric analysis of the AMPK signal normalized against the α-tubulin during

capacitation (A) and incubation (B). NCM, sperm incubated in non-capacitation medium; CM, sperm

incubated in capacitation medium; CM+CC, sperm incubated in capacitation medium with Compound

C; CM+A, sperm incubated in capacitation medium with A23817; CM+A+CC, sperm incubated in

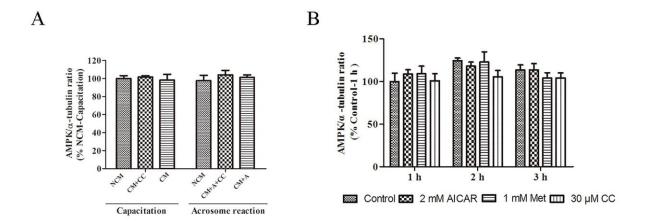capacitation medium with Compound C and A23817.

Fig.S4. Effects of AMPK inhibitor on sperm mitochondrial membrane potentials (ψm) during capacitation and acrosome reaction process. NCM, CM, CM+CC, CM+A and CM+A+CC treatments were the same with Fig. S3.
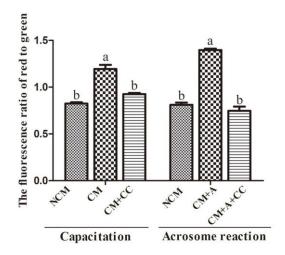

Fig.S5. Effects of AMPK modulators (AICAR, Met, and Compound C) on sperm, membrane integrity (A), acrosome reaction (B) and mitochondrial membrane potentials (ψm) (C) during 3 h of incubation.

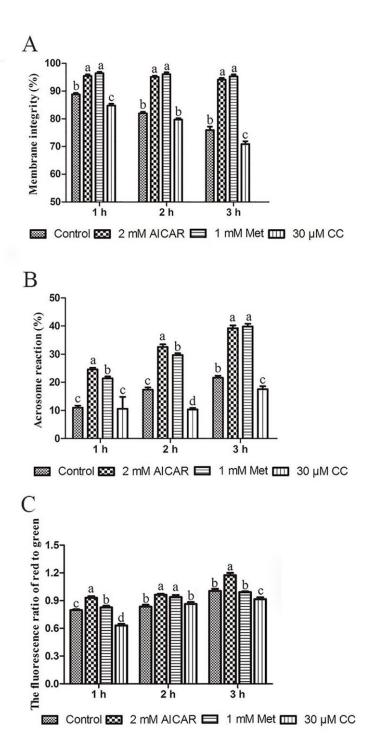Fig.S6. Effects of AMPK inhibitor on sperm lactate content (A), ATP content (B), pyruvate kinase activity (C) and LDH activity (D) during induction of capacitation and acrosome reaction in vitro. NCM, CM, CM+CC, CM+A and CM+A+CC treatments were the same with Fig. S3. Bars represent mean values ± SEM (n = 3 independent replicates). Different lower-case letters denote significant differences (p < 0.05).
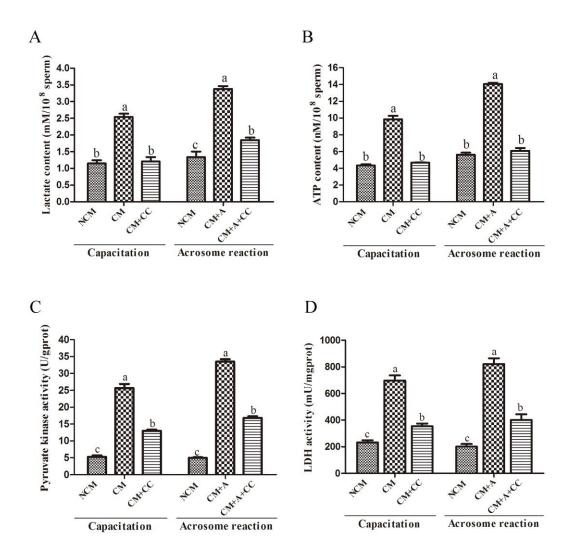
A — Lactate content (mM/10^8 sperm), B — ATP content (nM/10^8 sperm), C — Pyruvate kinase activity (U/gprot), D — LDH activity (mU/mgprot). Groups: NCM, CM, CM+CC (Capacitation); NCM, CM+A, CM+A+CC (Acrosome reaction).

Table 1. Effect of AMPK inhibitor on motility parameters during capacitation

Sperm parameters	NCM	CM	CM+CC
TM (%)	85.7 ± 0.9[b]	88.7 ± 0.8[a]	87.2± 0.8[ab]
PM (%)	54.8 ± 0.7[c]	71.7 ± 0.8[a]	60.7 ± 1.6[b]
BCF (Hz)	23.5 ± 0.6[c]	28.6 ± 0.2[a]	25.1 ± 0.3[b]
VCL (μm/s)	103.6 ± 0.7[c]	138.9 ± 1.7[a]	110.8 ± 2.3[b]
VSL (μm/s)	33.6 ± 1.6[c]	48.1 ± 1.1[a]	40.7± 1.8[b]
VAP (μm/s)	35.2 ± 0.7[c]	50.5 ±1.4[a]	37.3 ± 0.7[b]
WOB (%)	31.4 ± 0.7[b]	34.0 ± 0.8[a]	31.3 ± 0.3[b]
LIN (%)	29.2 ± 1.1[b]	36.7 ± 0.9[a]	34.9 ± 1.5[a]

Values are expressed as mean ± SEM. Different letters within column indicate significant difference (P < 0.05). TM, total motility; PM, progressive motility; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; BCF, beat-cross frequency; LIN, linearity (VSL/VCL); WOB, wobble (VAP/VCL); CC (Compound C), AMPK inhibitor.

Table 2. Effect of AMPK activators and inhibitor on motility parameters during incubation

Sperm parameters	1 h				2 h				3 h			
	Control	AICAR	Met	CC	Control	AICAR	Met	CC	Control	AICAR	Met	CC
TM (%)	90.1±1.5[a]	88.3±0.8[a]	87.8±0.3[a]	86.6±1.9[a]	86.7±0.8[a]	86.5±1.0[a]	86.2±1.9[a]	84.5±1.0[b]	83.7±0.7[b]	86.7±0.8[a]	85.1±1.6[a]	80.4±2.5[b]
PM (%)	65.2±2.0[b]	74.4±0.8[a]	73.1±0.9[a]	59.9±2.4[c]	54.8±1.2[b]	73.3±1.1[a]	73.0±2.1[a]	44.5±2.2[c]	41.8±0.7[c]	66.9±1.3[b]	72.4±1.5[a]	34.9±1.1[d]
BCF(Hz)	18.3±1.0[b]	27.3±0.9[a]	26.1±0.9[a]	15.5±0.4[c]	15.4±0.6[b]	25.9±1.1[a]	23.7±1.0[a]	13.2±1.0[c]	13.1±0.5[b]	25.6±1.7[a]	24.1±0.8[a]	9.1±0.3[c]
VCL(μm/s)	122.9±1.5[b]	139.8±3.1[a]	138.5±4.0[a]	112.8±1.5[c]	104.4±0.8[b]	136.0±1.7[a]	136.7±6.3[a]	77.9±5.0[c]	75.2±1.5[b]	128.9±5.9[a]	128.2±2.5[a]	66.9±4.0[c]
VSL(μm/s)	50.5±1.7[b]	61.5±2.7[a]	60.3±2.7[a]	49.8±1.4[b]	30.1±1.1[b]	51.6±1.3[a]	55.9±3.3[a]	24.0±1.4[c]	22.2±0.8[b]	46.5±3.0[a]	51.5±1.0[a]	20.0±2.3[b]
VAP(μm/s)	33.5±1.0[b]	50.7±2.1[a]	48.9±2.3[a]	28.6±0.5[c]	36.1±0.4[b]	47.6±1.8[a]	45.2±3.2[a]	25.6±1.7[c]	25.3±0.5[b]	43.6±3.3[a]	42.5±1.8[a]	20.4±1.0[c]
WOB (%)	24.1±2.6[b]	34.1±1.1[a]	33.0±0.5[a]	21.7±1.5[b]	32.3±2.2[a]	32.5±1.1[a]	30.0±1.2[a]	27.9±1.7[b]	27.7±1.8[b]	30.8±1.7[a]	30.7±1.0[a]	24.1±1.0[c]
LIN (%)	41.0±1.1[a]	44.0±1.3[a]	43.5±1.1[a]	44.2±1.0[a]	26.8±0.8[b]	40.1±1.4[a]	43.4±0.9[a]	24.0±1.0[b]	22.6±0.4[b]	38.0±2.7[a]	42.0±1.8[a]	21.3±1.7[b]

Values are expressed as mean ± SEM. Different letters within column indicate significant difference ($P < 0.05$). TM, total motility; PM, progressive motility; VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity; BCF, beat-cross frequency; LIN, linearity (VSL/VCL); WOB, wobble (VAP/VCL); AICAR, Met (metformin): AMPK activators; CC (Compound C), AMPK inhibitor.