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Research Article

Transient exposure to sodium butyrate after germinal vesicle breakdown improves meiosis but not developmental competence in pig oocytes

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Abstract

Oocyte maturation is a complex process during which epigenetic modifications are dramatically changed, especially histone acetylation and phosphorylation. We have investigated the effects of NaBu (sodium butyrate), a natural HDAC (histone deacetylase) inhibitor, on porcine oocyte maturation at different stages and subsequent embryonic development to improve IVF (*in vitro* fertilization) and embryo production. COCs (cumulus oocyte complexes) were cultured, IVM (*in vitro* maturation) supplemented with 1 mM NaBu before or after GVBD [GV (germinal vesicle) breakdown] during maturation. NaBu delayed oocyte meiosis in the GV and GVBD stages in an exposure-dependent manner. However, the short treatment with 1 mM NaBu after GVBD significantly improved the meiotic competence. No positive effects of NaBu on GSH levels and subsequent embryonic development following IVF were seen. Transient exposure to NaBu after GVBD improves meiotic competence, but not subsequently, probably by having an effect on histone acetylation during oocyte maturation.

Keywords: developmental competence; histone acetylation; *in vitro* maturation; porcine oocyte; sodium butyrate

1. Introduction

Oocyte IVM (*in vitro* maturation) is the basis of assisted reproduction, making improvements in oocyte quality and developmental competence important in SCNT (somatic cell nuclear transfer) and in producing transgenic animals. Meiosis stage-dependent histone modifications are crucial in oocyte development. Covalent histone modification is involved in the regulation of acetylation, methylation, phosphorylation, ubiquitination and sumoylation (Kouzarides, 2007). Acetylation affects many fundamental processes and an increase in histone acetylation is always followed by cell-cycle arrest, differentiation or apoptosis (Senese et al., 2007; Takai et al., 2008; Frew et al., 2009). During oocyte IVM, histone acetylation and phosphorylation are dramatically altered (Wang et al., 2006). After resumption of meiosis, the signal for the acetylation of H3K14 decreases and remains at low level. In diakinesis, both the H3K14 and H3K18 signals disappear completely in MI (metaphase I) at 27 h of IVM. The acetylated histone H3K9 appears at low level at TI (telophase I). Similarly, the acetylation of H3K14 temporarily appears around AT-I (anaphase I and telophase I). These acetylation signals then disappear completely when the oocytes reaches MII (Bui et al., 2007). We found that the histones were re-acetylated when the oocytes progressed through MI in sheep (Tang et al., 2007), mouse (Akiyama et al., 2004) and pig (Endo et al., 2005; Xue et al., 2010). Also acetylated histone H3K18 and H3K23 at meiotic stages from GVBD [GV (germinal vesicle) breakdown] to MI was established (Xue et al., 2010). It was hypothesized that intervention with an HDAC (histone deacetylase) inhibitor may increase meiotic and developmental competence.

NaBu (sodium butyrate) is a non-toxic, 4-carbon fatty acid synthesized naturally by anaerobic fermentation of carbohydrates in the colon, acting as a potent HDAC inhibitor (Sealy and Chalkley, 1978). It is a non-competitive inhibitor of HDAC that is not associated with the substrate-binding site (Cousens et al., 1979); the binding site and mechanism by which NaBu inhibits HDAC activity remain unknown. It can induce potent cellular effects *in vitro* in a range of cell lines, including growth arrest and differentiation (Kumar et al., 2007; Lee et al., 2009). In different cellular systems, NaBu can inhibit not only histone deacetylation, but histone phosphorylation and DNA hypermethylation (de Haan et al., 1986; Parker et al., 1986; Boffa et al., 1994). However, the effect of NaBu on porcine oocyte IVM is unclear.

We have analysed the role of NaBu on porcine oocyte meiosis and subsequent embryonic developmental competence to improve IVF (*in vitro* fertilization) and *in vitro* embryo production. Alteration of histone acetylation by NaBu on oocyte quality during IVM, and the levels of AcH3K14 (acetylation of Lys^{14} on histone 3) of the oocytes have been investigated. Oocyte GSH content, the IVF status and the spindle status were also examined.

Abbreviations: AcH3K14, acetylation of Lys^{14} on histone 3; AT-I, anaphase I and telophase I; COC, cumulus oocyte complex; DK, diakinesis; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GV, germinal vesicle; GVBD, GV breakdown; HDAC, histone deacetylase; MI, metaphase I; MII, metaphase II; IVF, *in vitro* fertilization; IVM, *in vitro* maturation; MPN, male pronuclear formation; mTBM, modified Tris-buffered medium; NaBu, sodium butyrate; PFA, paraformaldehyde; PVA, polyvinyl alcohol; TSA, trichostatin A.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich Chemical Company, unless stated otherwise.

2.2. Oocyte collection and IVM

Porcine ovaries from a local abattoir were taken to the laboratory in PBS (35–39°C) within 2 h. The ovaries were washed thrice in sterile PBS. Follicular fluid and COCs (cumulus oocyte complexes) were aspirated from 2 to 8 mm antral follicles with a 10 ml disposable syringe with an 18-gauge needle and were expelled into sterile Petri dishes (9 cm diameter) at 39°C. COCs with >3 layers of intact and compact cumulus were selected, washed thrice in manipulation fluid [TCM199 supplemented with 0.1% PVA (polyvinyl alcohol)] and cultured in IVM media [TCM-199 supplemented with 26 mM sodium bicarbonate, 3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 10 ng/ml epidermal growth factor, 0.5 unit/ml porcine LH (luteinizing hormone), 0.5 unit/ml porcine FSH (follicle-stimulating hormone), 0.1% (w/v) PVA, 10% fetal calf serum, 75 mg/ml penicillin G and 50 mg/ml streptomycin]. A group of 30 oocytes were cultured in a 100 μl drop of maturation medium for up to 44 h at 39°C in a humid atmosphere of 5% CO_2 in air. All maturation media drops containing oocytes were covered with a thin layer of mineral oil and incubated in pre-equilibrated culture medium.

2.3. NaBu treatment

Preliminary work showed an effective concentration of NaBu had little toxicity at 1 mM on somatic cells (Kumar et al., 2007; Yang et al., 2007), which was chosen as the standard dose. NaBu solution prepared in PBS was diluted in maturation medium to final concentrations of 1, 5 and 10 mM. To explore the role of NaBu in meiotic resumption, the oocytes were cultured and treated with 1 mM NaBu for either 2 h or 24 h in maturation medium. To follow the effects of NaBu on meiotic progression through MI to MII (metaphase II), oocytes were cultured for 24 h and subsequently treated with NaBu for a short time (24 h+NaBu 2 h) or a long time (24 h+NaBu 20 h). PBS at 0.1% was used as the control. After 44 h of maturation, oocytes were freed of cumulus cells by treatment with 0.1% hyaluronidase and repeated pipetting. Cumulus-free oocytes were used in the following experiments.

2.4. IVF and embryo culture

At the end of the maturation period, each group of matured oocytes was washed gently until cumulus cells were removed with a pipette and oocytes were transferred to drops of IVF media (20 oocytes/100 μl of media). IVF medium was mTBM (modified Tris-buffered medium) containing 113.1 mM NaCl, 3.0 mM KCl, 7.5 mM $CaCl_2{\cdot}2H_2O$, 20.0 mM Tris, 11.0 mM glucose and 5.0 mM sodium pyruvate supplemented with 2 mg/ml BSA and 2 mM caffeine (Herrick et al., 2003), which was used following preparation and equilibration overnight, as above. Semen supplied by a porcine artificial insemination centre was stored at 17°C for up to 7 days. The pellet was placed on a 45/90 Percoll gradient and centrifuged at 300 ***g*** for 30 min before being resuspended in DPBS (Dulbecco's PBS, 5 volumes) supplemented with 1 mg/ml BSA Fraction V (Sigma), 100 g/ml penicillin G and 75 g/ml streptomycin sulfate and washed twice to remove the Percoll at 200 ***g*** for 10 min. Afterwards, 1 ml of mTBM was gently added to the pellet. The top layer of mTBM containing active spermatozoa was removed 10 min later and applied to the oocyte microdrops, giving a final concentration of 1×10^5 cells/ml and a final oocyte/spermatozoa ratio of 1:5000. After 6 h, the presumptive zygotes were washed twice in PZM3 (Okada et al., 2006) and transferred to PZM3 (20 oocytes/100 μl) for further culturing at 39°C in 5% CO_2 in air until day 7.

2.5. Evaluation of nuclear maturation and pronuclear formation

Oocytes after 44 h of IVM, putative zygotes after 10 h of insemination and embryos after *in vitro* culture for 7 days were fixed in 4% (w/v) PFA (paraformaldehyde) for 20 min after removal of the oocyte zona pellucida with 0.05% pronase in PBS. The samples were stained with 10 μg/ml Hoechst 33342 for 20 min and were mounted on to glass slides. Nuclear maturation and pronuclear location were determined using an epifluorescence microscope. Embryos on to day 7 (the day of fertilization being defined as day 0) were assessed for blastocyst formation rate and cell number.

2.6. Analysis of oocyte histone acetylation level and spindle status

After their zona pellucida had been removed with 0.05% pronase in PBS, oocytes in different groups were fixed with 4% PFA in PBS (pH 7.4) for 20 min at room temperature and permeabilized with 1% Triton X-100 for at least 40 min. The oocytes were treated with blocking solution (PBS supplemented with 1% BSA) for 20 min and incubated overnight at 4°C with an anti-AcH3K14 primary antibody (Upstate) diluted at 1:200. After being washed thrice in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 (washing solution) for 10 min each wash, they were incubated with secondary FITC-conjugated antibody (Santa Cruz Biochemistry) diluted at 1:200 for 1 h at 37°C, and then stained with 10 μg/ml Hoechst 33342 for 20 min. Following extensive washing, all samples were mounted with a coverslip supported by 4 columns of a mixture of petroleum jelly and paraffin (9:1) and examined with a confocal laser-scanning microscope (Olympus Fv100) as quickly as possible. As a negative control, all groups of oocytes were incubated with non-immune rabbit serum instead of rabbit polyclonal primary antibody. Each experiment was repeated thrice, and at least 30 oocytes were examined each time.

To determine the spindle status during IVM, oocytes at 36 h of maturation were fixed with 4% PFA in PBS (pH 7.4) for 20 min at room temperature after removing their zona pellucida. They were permeabilized with 1% Triton X-100 for at least 40 min and treated with blocking solution (PBS supplemented with 1% BSA) for

20 min and incubated with fluorescently labelled anti-α-tubulin antibody (Sigma) diluted at 1:200 at 37°C for 1 h. After being washed thrice in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 (washing solution) for 10 min each wash, the oocytes were stained with 10 μg/ml PI (propidium iodide) for 10 min. After extensive washing, they were mounted and examined as described above. Each experiment was repeated thrice, and at least 40 oocytes were examined each time.

2.7. Measurement of intracellular GSH content

Intracellular GSH content was measured as described by Lee et al. (2005) and Viet Linh et al. (2009). For each replicate, pools of 30–50 matured oocytes were placed in 5 μl of 0.2 M sodium phosphate buffer containing 10 mM Na_2–EDTA (pH 7.2) and 5 μl of 1.25 M phosphoric acid in microtubes, and then frozen at −80°C. The concentration of GSH in the oocytes was determined by DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)]–GSSG recycling assay. Briefly, the samples were thawed and 175 μl of sodium phosphate buffer containing 0.33 mg/ml NADPH (Sigma), 25 μl of 6 mM DTNB (Wako) and 40 μl of water was added to each sample tube. The samples were warmed at room temperature for 15 min, and the assay was initiated by the addition of 5 μl of 125 units glutathione disulfide reductase (Sigma). Absorbance was measured five times (DU7500; Beckman Coulter) at 30 s intervals at a wavelength of 415 nm by spectrophotometry. A GSH standard and sample blank lacking GSH were also assayed. Standards were prepared for each assay, and the total GSH content per sample was determined from a standard curve of GSH. The GSH concentration per oocyte was calculated by dividing the total concentration per sample by the number of oocytes present in the sample.

2.8. Statistical analysis

Three replicate trials were conducted for each treatment, and the mean percentage (±S.E.M.) was calculated for each experimental group. Data were analysed by one-way ANOVA using SPSS software (Statistics Production for Service Solution, Version 16.0) after being transformed via LSD (least significant difference). statistical significance was taken as $P<0.05$.

3. Results

3.1. Nuclear maturation of porcine oocytes during *in vitro* culture

We found that most oocytes were at the GV stage (98.4%) at 0 h of culture (Figures 1 and 5); by 24 h, 90.1% of oocytes underwent GVBD and proceeded to the diakinesis stage (67.6%) and MI (22.5%). By 36 h, 46.7% of oocytes were in AT-I stage. After 44 h, 73.6% of oocytes were at the MII stage, which was taken as nuclear maturation. These data obtained by Hoechst 33342 staining were crucial for the following experiments.

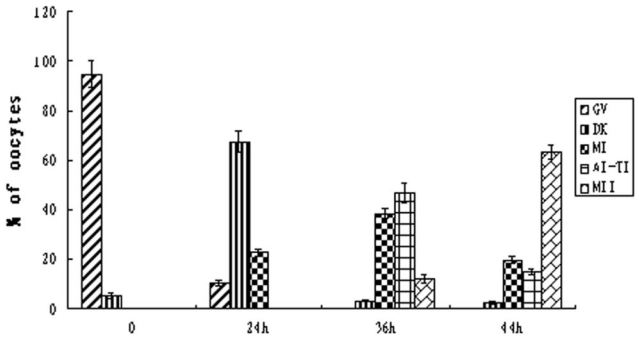

Figure 1 Nuclear maturation of porcine oocytes during *in vitro* culture
Experiments were repeated thrice for each time-point examined (DK, diakinesis).

3.2. Effects of NaBu on oocyte maturation in GV and GVBD stages

To determine the effect of NaBu in GV and GVBD stages, oocytes were treated with 1 mM NaBu for either 2 h or 24 h during IVM. Table 1 shows that NaBu significantly delayed oocyte IVM. Treatment with 1 mM NaBu at GV for only 2 h slightly improved the level of acetylation on H3K14 (Figures 2B, 2B′ and 2B′′) (73.3±6.7, $n=30$), but after 44 h maturation, 7.7% of the oocytes remained at GV and 29.9% were observed at MI. When cultured with 1 mM NaBu for 24 h, hyperacetylation on H3K14 occurred (Figures 2D, 2D′ and 2D′′) (100%, $n=30$), and after 44 h maturation, ~13% of oocytes were at GV, and more oocytes (47.2%) remained in MI.

3.3. Effects of NaBu on oocyte maturation after GVBD

To avoid the effects of NaBu on GVBD, 1 mM NaBu was added to the maturation medium to induce hyperacetylation after 24 h IVM for different times. At 26 h maturation, the signal of histone acetylation of control oocytes disappeared (Figure 2E), but histone deacetylation of oocytes treated with NaBu for only 2 h was strongly inhibited (100%, $n=30$; Figure 2F). After 44 h, nuclear maturation increased to 86% compared with control (74%). However, there was no difference in nuclear maturation rate between the long treatment of 24 h and control (Table 2). On the contrary, more aberrant chromosomes were observed compared with the control (28.5±5.7 versus 5.1±0.4, $P<0.05$; Figures 3 and 4).

We also evaluated the effect of transient exposure to NaBu at different concentrations of NaBu. Table 3 shows that maturation rate increased in relation to NaBu concentration, but a significant percentage of these oocytes degenerated (3.6% and 7.7% versus 0%; $P<0.05$).

3.4. Effects of transient exposure to NaBu on GSH

Cytoplasmic maturation of oocytes treated with NaBu for 2 h after GVBD was assessed from their GSH content, but did not increase in a corresponding manner to increased NaBu concentration (Table 4).

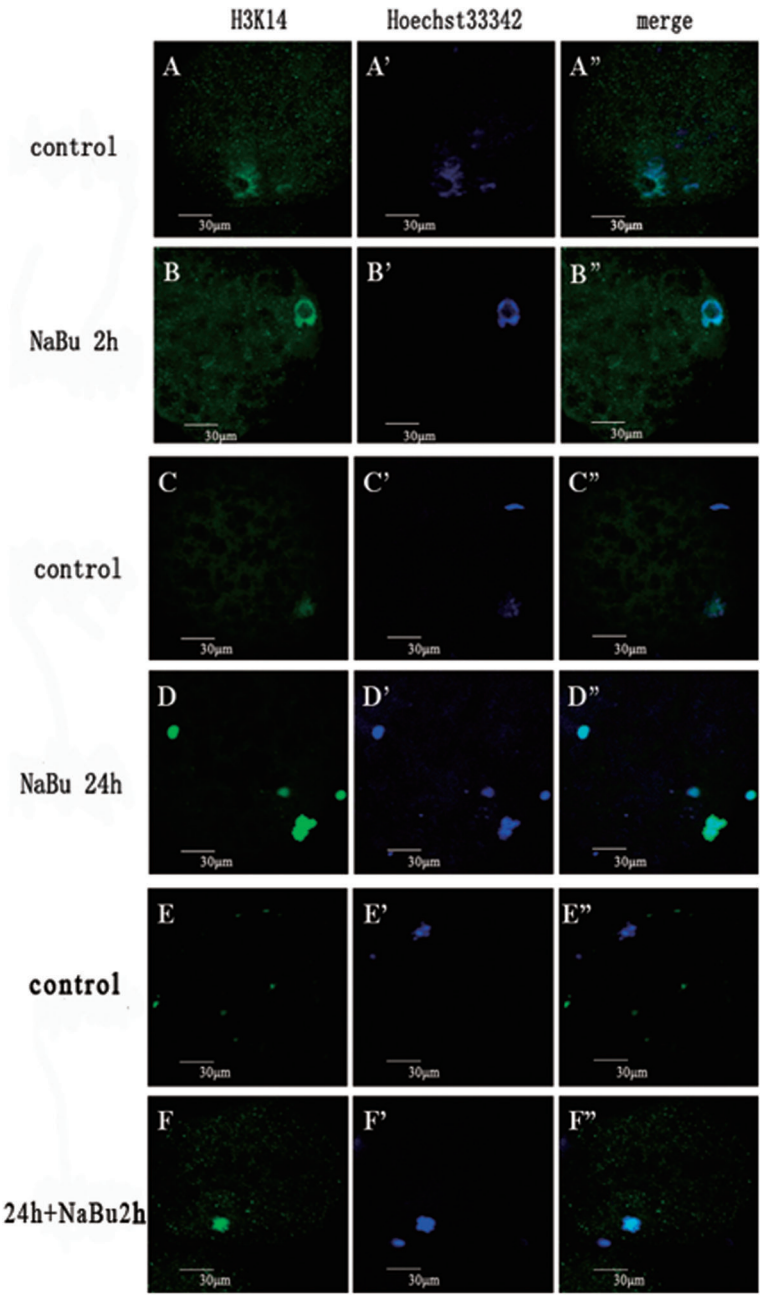

Figure 2 **Acetylation patterns of histone H3 Lys^{14} (H3K14) during porcine oocyte meiosis**
Oocytes were immunostained with anti-AcH3K14 antibody (green). DNA was counterstained with Hoechst 33342 (blue). Oocytes were cultured without NaBu for 2 h (**A**, **A′**, **A′′**); oocytes were cultured with 1 mM NaBu for 2 h (**B**, **B′′**, **B′′**); oocytes were cultured without NaBu for 24 h (**C**, **C′**, **C′′**); oocytes were cultured with 1 mM NaBu for 24 h (**D**, **D′**, **D′′**); oocytes were cultured for 26 h (**E**, **E′**, **E′′**); oocytes were cultured for 24 h and subsequently treated with 1 mM NaBu for 2 h (**F**, **F′**, **F′′**).

Table 1 **Effect of 1.0 mM NaBu on the oocytes maturation before GVBD after 44 h maturation**
Experiment was replicated five times. DK, diakinesis.

		% Oocytes (*n*)		
	No. of oocytes examined	GV	DK–MI	MII
Control	272	0 ± 0 (0)[a]	26.48 ± 2.24 (72)[a]	73.51 ± 2.25 (200)[a]
NaBu 0–2 h of IVM	339	7.65 ± 0.49 (26)[b]	29.85 ± 2.5 (100)[a]	59.50 ± 2.62 (203)[b]
NaBu 0–24 h of IVM	363	13.04 ± 1.08 (48)[c]	47.23 ± 1.27 (173)[b]	39.73 ± 0.25 (142)[c]

[a–c]Values with different superscripts are significantly different ($P<0.05$).

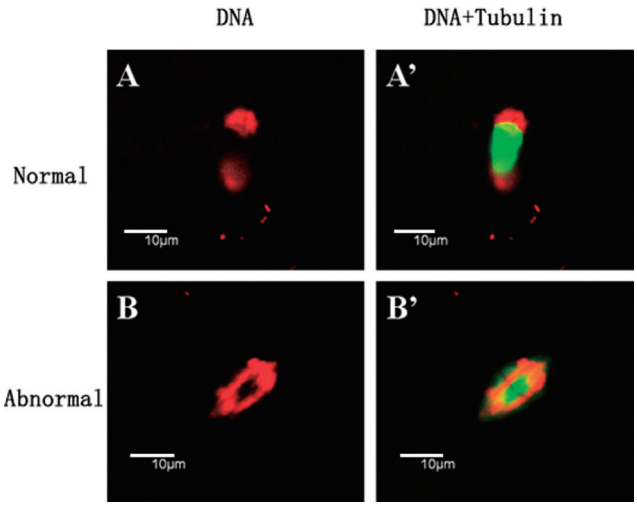

Figure 3 **Oocytes treated with NaBu produce chromosome segregation defects in AT-I oocytes**
Immunostaining of spindles with α-tubulin antibody (green) and chromosomes with PI (red) in normal (**A**, **A′**) and defective AT-I oocytes (**B**, **B′**).

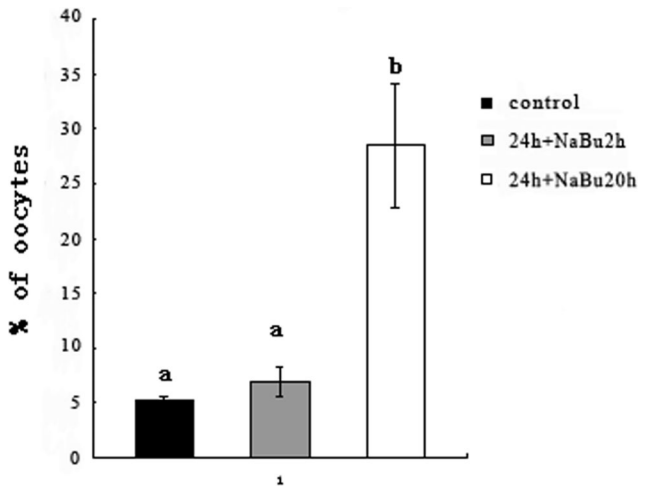

Figure 4 **Quantitative analysis of the induction of chromosome segregation defects after treatment with 1 mM NaBu after GVBD**
The graph shows the means ± S.D. of the results obtained in three independent experiments. Different superscripts indicate statistical differences ($P<0.05$).

Table 2 **Effect of 1 mM NaBu on the oocytes maturation through GVBD after 44 h maturation**
Experiment was replicated four times.

		% Oocytes (*n*)		
	No. of oocytes examined	GV	LD–MI	MII
Control	227	0 ± 0 (0)	26.04 ± 4.47 (65)[a]	73.95 ± 5.27 (162)[a]
24 h+NaBu 2 h	203	0 ± 0 (0)	13.97 ± 3.47 (32)[b]	86.03 ± 3.07 (171)[b]
24 h+NaBu 20 h	172	0 ± 0 (0)	22.19 ± 3.05 (37)[a]	77.81 ± 3.02 (135)[a]

[a,b]Values with different superscripts are significantly different ($P<0.05$).

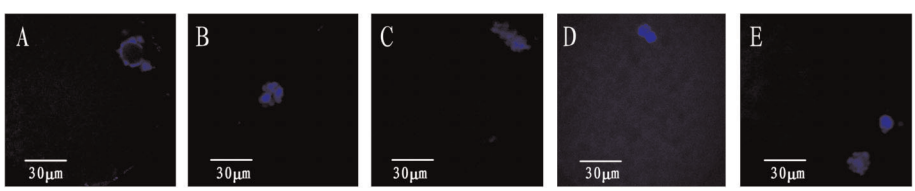

Figure 5 **Changes in oocyte nuclear maturation**
DNA was stained with Hoechst 33342 (blue). (**A**) Germinal vesicle, (**B**) diakinesis, (**C**) metaphase I, (**D**) anaphase I and telophase I, and (**E**) metaphase II.

Table 3 **Effect of transient exposure (2 h) of different concentration of NaBu after GVBD on pig oocytes**
Experiment was replicated four times.

		% Oocytes (*n*)			
NaBu (mM)	No. of oocytes examined	GV	DK–MI	MII	Degenerated
Control	227	0 ± 0 (0)	21.96 ± 5.55 (54)[a]	74.18 ± 2.35 (167)[a]	0 ± 0 (0)[a]
1	203	0 ± 0 (0)	14.40 ± 2.54 (30)[b]	85.60 ± 0.66 (173)[b]	0 ± 0 (0)[a]
5	204	0 ± 0 (0)	8.44 ± 2.72 (19)[c]	87.92 ± 3.07 (177)[b]	3.64 ± 0.79 (8)[b]
10	203	0 ± 0 (0)	2.73 ± 1.36 (5)[d]	89.60 ± 2.41 (181)[b]	7.67 ± 2.28 (17)[c]

[a–d]Values with different superscripts are significantly different ($P<0.05$).

Table 4 **Glutathione concentration in porcine MII oocytes transient exposure to different concentrations of NaBu after GVBD during IVM**
Experiment was replicated four times, means ± S.E.M. are presented.

NaBu (mM)	Glutathione concentration* (pmol/oocyte)
0	8.27 ± 0.2[a]
1.0	7.19 ± 1.2[a]
5.0	6.16 ± 0.2[a]
10.0	4.87 ± 0.6[b]

* The glutathione concentrations of IVM oocytes were measured after 44 h of IVM.
[a,b]Values with different superscripts are significantly different ($P<0.05$).

3.5. Fertilization status after IVF and *in vitro* development of IVF embryos

Following increased NaBu concentration, the rates of sperm penetration, monospermic fertilization and MPN (male pronuclear formation) formation were no different from controls (71.0–69.9, 58.7–39.6 and 30.8–33.9% respectively; Tables 5 and 6). The rates of cleavage and blastocyst formation of *in vitro* culture after 2 and 7 days also did not differ from the controls (56.6–50.9 and 16.5–15.4% respectively), nor did the number of cells in blastocysts.

4. Discussion

NaBu delayed porcine oocyte meiosis in proportion to the length of exposure, but transient exposure to NaBu after GVBD improved the meiotic process. The delay of meiosis induced by NaBu is similar to that of TSA (trichostatin A), another HDAC inhibitor. Wang et al. (2006) reported that inhibition of histone deacetylation with TSA delayed the onset of GVBD. Complete inhibition of GVBD was also observed in the results of Bui et al. (2007). However, Kim et al. (2003) found no meiotic blockage in TSA-treated mouse oocytes, possibly due to interspecies differences. Endo et al. (2005) also found that treatment with 100 nM TSA had no effect on the maturation rate of porcine oocytes. This lack of effect may be partially explained by different culture conditions. Bui et al. (2007) cultured the COCs with a piece of granulosa tissue, which may have enhanced the effect of TSA. Oocyte maturation can be inhibited by granulosa cells (Schulz et al., 1985), but results from Wang et al. (2006) showed TSA affects meiotic resumption by direct action on the oocytes rather than through the pathway mediated by cumulus cells. The longer treatment of 24 h with NaBu in GVBD in our experiments increased the level of histone acetylation and significantly inhibited the maturation rate of the oocyte. Although shorter treatment (2 h) with NaBu in the GV stage did not improve the level of histone acetylation, it may be that the hyperacetylation of histones in the GV stage (in which the maturation of oocytes was delayed significantly) was due to a higher oocyte sensitivity to NaBu. We observed that 2 h treatment with NaBu after GVBD significantly increased the maturation rate. Previous data showed that histones were acetylated again when the oocytes progressed through MI (Endo et al., 2005). The resumption of meiosis is accompanied by extensive histone deacetylation, and the reversal of histone acetylation at MI is typical of maturation. Treatment with NaBu after GVBD counteracted initial spontaneous histone deacetylation, which might have helped the reversal of histone acetylation and improve the meiotic competence of oocytes. Petr et al. (2009) found that TSA treatment after GVBD improves the meiotic competence in maturing growing oocytes. However, 24 h treatment with NaBu after GVBD did not improve the maturation rate compared with the control group, possibly due to the high frequency of abnormal chromosome segregation in oocytes at AT-I. The change of histone acetylation shows spatiotemporal specificity during oocyte maturation. An increase of histone acetylation in oocytes in an inappropriate period will produce chromosome deficiency, and may result in aneuploid embryo formation, which causes early embryo death, spontaneous abortion and genetic disease. Maintaining hyperacetylation leads to a high frequency of lagging chromosomes and chromatin bridges in AT-I oocytes (Wang et al.,

Table 5 **IVF of porcine oocytes after IVM transient exposure to different concentrations of NaBu**
Experiment was replicated three times. Means ± S.E.M. are presented.

NaBu (mM)	No. of oocytes examined	% oocytes penetrated by sperm (*n*)	% oocytes with MPN (*n*)	% monospermic oocytes (*n*)
0	97	71.03 ± 3.90 (68)	58.74 ± 5.70 (40)	29.81 ± 4.12 (30)
1.0	84	69.89 ± 6.42 (57)	39.61 ± 2.86 (23)	33.90 ± 6.05 (27)
5.0	95	71.69 ± 8.18 (67)	41.75 ± 11.53 (27)	31.54 ± 10.62 (29)
10.0	88	72.53 ± 7.93 (62)	44.58 ± 10.23 (26)	31.37 ± 4.84 (29)

Table 6 **Transient exposure to different concentrations of NaBu during IVM after GVBD on subsequent embryonic development following IVF of porcine oocytes**
Experiment was replicated four times.

		% embryos developed (*n*)		
NaBu (mM)	No. of oocytes examined	Cleavage on day 2	Blastocyst on day 7	Cell number in blastocyst mean ± S.E.M. (*n*)
0	258	56.6 ± 4.3 (146)	19.5 ± 5.5 (31)	48.2 ± 2.5 (7)
1.0	259	50.9 ± 3.6 (131)	17.4 ± 4.6 (25)	45.3 ± 3.2 (8)
5.0	223	47.3 ± 7.4 (113)	15.9 ± 3.1 (20)	43.6 ± 1.7 (6)
10.0	290	48.7 ± 4.2 (139)	16.6 ± 3.1 (24)	47.2 ± 3.9 (8)

2006). Akiyama et al. (2006) reported that inadequate histone deacetylation during oocyte meiosis causes aneuploidy and embryo death in mice. The high ratio of abnormal chromosomes segregation was also observed in our experiments on oocytes with the longer NaBu treatment, but no differences were found between the short treatment group and the control group.

NaBu has been an essential agent for determining the role of histone acetylation in chromatin structure and function, which results in multiple effects on cultured mammalian cells, including the inhibition of proliferation, induction of differentiation and induction or repression of gene expression (Kumar et al., 2007; Li et al., 2008; Lee et al., 2009; Das et al., 2010). The promoters of butyrate-responsive genes have response elements; butyrate action is often mediated through Sp1-/Sp3-binding sites (Walker et al., 2001; Pan et al., 2010). Although the inhibition of HDAC activity affects the expression of only 2% of mammalian genes (Mariadason et al., 2000), for most biological processes a larger set of genes are induced compared with the number with decreased expression by NaBu according to microarray analysis in HIEC (human intestinal epithelial cell) line (Blais et al., 2007). However, in oocyte maturation, the effects of NaBu on cytoplasmic maturation of oocytes remained unknown. Hence, in the present study, the developmental competence of the oocytes given 2 h treatment with NaBu after GVBD were determined by GSH content and the status of IVF. GSH plays an important role in protecting cells against oxidative damage (Meister, 1983). The intra-oocyte concentration of GSH is considered an important indicator of cytoplasmic maturation (Yoshida et al., 1993). The result shows that short treatment with NaBu did not affect the GSH content of the oocytes, in agreement with MPN formation and the cleavage and blastocyst rate in IVF. Although the elevation of histone acetylation strongly influences nuclear maturation, this effect is not observed in cytoplasmic maturation, which is mainly because of transcription repression during oocyte maturation (De La Fuente, 2006). Another reason that the inhibition of HDACs has different effects on meiotic oocyte maturation and mitotic cells of the embryo is that HDACs are localized differently in oocytes and the early stages of the embryo (Endo et al., 2008).

In conclusion, treatment with NaBu increased histone acetylation of the porcine oocyte, but delayed maturation relative to the length of treatment in GV and GVBD. However, transient exposure to NaBu after GVBD improved meiotic competence, but not developmental competence. These results may be useful for *in vitro* embryo production.

Author contribution

Limei Liu performed research, analysed data and wrote the paper; Guangqi Song performed research and analysed data; Fei Gao performed research and analysed data; Jiyu Guan analysed data; Bo Tang designed research and analysed data, Ziyi Li designed research, analysed data and wrote the paper.

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