

# Phosphorylation of histone H3 on Ser-10 by Aurora B is essential for chromosome condensation in porcine embryos during the first mitotic division

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**Abstract** Phosphorylation of histone H3 on Ser-10 (H3S10ph) is involved in regulating mitotic chromosome condensation and decondensation, which plays an important regulatory role during mitotic cell cycle progression in mammalian cells. However, whether H3S10ph plays a similar role in early porcine embryos during the first mitotic division remains uncertain. In this study, the subcellular localization and possible roles of H3S10ph were evaluated in the first mitotic cell cycle progression of porcine embryos using western blot, indirect immunofluorescence and barasertib (H3S10ph upstream regulator Aurora-B inhibitor) treatments. H3S10ph exhibited a dynamic localization pattern and was localized to chromosomes from prometaphase to anaphase stages. Treatment of porcine embryos with barasertib inhibited mitotic division at the prophase stage and was associated with a defect in chromosome condensation accompanied by the reduction of H3S10ph. These results indicated that H3S10ph is involved in the first mitotic division in porcine embryos through its regulatory function in chromosome condensation, which further affects porcine embryo cell cycle progression during mitotic division.

**Keywords** Pig · Embryos · Mitotic division · H3S10ph · Chromosome condensation

## Introduction

The first mitotic division in embryos is a special mitotic cell cycle process that transitions the oocytes from meiosis (Clift and Schuh 2013) and is characterized by a prolonged duration compared with subsequent divisions (Hormanseder et al. 2013). The process of the first mitotic division in embryos is regulated by a network of proteins and involves numerous biological events, such as chromosome condensation, bipolar spindle formation, chromosome alignment and segregation (Nurse 2000). Chromosome condensation is the first event that occurs at the start of embryo mitotic division, which is essential for the packaging of chromatin fibers into chromosomes and correct separation. However, the mechanism of chromatin condensation is still not fully clear. The condensed chromosome fiber consists of a repetitive unit termed the nucleosome. Every nucleosome contains an octamer (two each of H2A, H2B, H3, and H4) wrapped by 146 base pairs of DNA (Arents et al. 1991). The N termini of the core histones are essential for mitotic chromosome compaction (Barre et al. 2001). The phosphorylation of histone H3 on Ser-10 (H3S10ph) is thought to play a key role in mitotic chromosome condensation in many organisms (Prigent and Dimitrov 2003; Hendzel et al. 1997; Nowak and Corces 2004; Wei and Allis 1998). However, in pig oocytes, interestingly, histone H3 phosphorylation on Ser-10 is not required for meiotic chromosome condensation (Jelinkova and Kubelka 2006; Ju et al. 2016). It is not clear whether it is required for the subsequent embryo mitotic chromosome condensation process during the first mitotic division in porcine embryos.

Aurora-B, a member of a highly conserved serine/threonine protein kinase family (Carmena and Earnshaw 2003; Carmena et al. 2012), is responsible for the phosphorylation of the Ser-10 of histone H3 during mitotic division. In

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*Drosophila*, cell depletion of Aurora-B kinase is associated with reduction of histone H3 phosphorylation and only partial chromosomes condensation at mitosis (Giet and Glover 2001). Aurora-B H3 kinase is required for H3S10ph during meiosis and mitosis in *S. cerevisiae* and *C. elegans* (Hsu et al. 2000). Histone H3 was phosphorylated on Ser-10 directly by Aurora-B in *Xenopus* egg cell-free extracts during mitosis (Murnion et al. 2001). In mammalian cells, Aurora-B is responsible for histone H3 phosphorylation on Ser-10 during mitosis (Goto et al. 2002), and glutathione S-transferase (GST) pull-down experiments also revealed that Aurora-B could physically interact with the H3 N-terminal tail and efficiently phosphorylate Ser-10 in HeLa cells (Crosio et al. 2002). The above data indicate that Aurora B kinase produces H3S10ph to regulate the activity of histone H3 on Ser-10.

In this study, we examined the correlation between H3S10ph and chromosome condensation in porcine embryos during the first mitotic division using the Aurora-B inhibitor barasertib (Yang et al. 2007; Azzariti et al. 2011; Sessa and Villa 2014; Mortlock et al. 2007; Alferez et al. 2012). The results indicate that barasertib treatment inhibits mitotic division at the prophase stage and is associated with defects in chromosome condensation and the reduction of H3S10ph. These results indicated that H3S10ph is involved in the first mitotic division in porcine embryo through its regulatory function in chromosome condensation, which further affects the cell cycle and first mitotic division in porcine embryos.

## Materials and methods

### Antibodies and reagents

The rabbit polyclonal anti-histone H3 (phospho S10) antibody was obtained from Abcam (Cambridge, UK). Barasertib was procured from Selleck Chemicals. The mouse monoclonal anti- $\alpha$ -tubulin-FITC antibody was from Sigma (St. Louis, MO, USA). Rabbit polyclonal anti- $\beta$ -tubulin antibody was obtained from Yi Fei Xue Bio Tech (NanJing, China). The rhodamine (TRITC)-conjugated goat anti-rabbit IgG (H+L) was from ZSGB-BIO (Beijing, China). All other chemicals and agents used in different experiments were bought from Sigma-Aldrich (USA) unless otherwise stated.

### Culture and activation of porcine oocytes

Oocytes were aspirated from 3- to 6-mm-diameter antral follicles with a 10-mL disposable syringe. After three times washing with TCM199 medium (Gibco BRL, Gaithersburg, MD, USA), oocytes with at least three layers of

intact cumulus cells and even ooplasm were chosen for maturation culture in four-well plates (Nunclon, Roskilde, Denmark) (one well containing approximately 80 oocytes in 500  $\mu$ L of maturation culture medium) (Lai and Prather 2003). Oocytes were cultured for up to 44 h at 38.5 °C in saturated humidified atmosphere of 5% CO<sub>2</sub>. After maturation, oocytes were separated from cumulus cells by 0.1% hyaluronidase at 37 °C for 5 min. The denuded oocytes with an identical ooplasm, unbroken cytoplasmic membrane, and visible first polar body (pbI) were regarded at the MII stage. For activation, these MII stage oocytes were selected and equilibrated in activation medium (0.3 M mannitol, 1 mM CaCl<sub>2</sub>, 0.1 mM MgCl<sub>2</sub> and 0.1% BSA) for 3 min. Then, the oocytes were activated with a single direct current (DC) pulse of 1.5 kV/cm for 80  $\mu$ s using an Electro-cell Manipulator (CRY-3; Ningbo Xinzhi Co., Ltd., Ningbo, China). After activation, oocytes were washed thrice and then transferred to porcine zygote medium 3 (PZM-3) (Lai and Prather 2003) and incubated for 2 h, then the barasertib was added to the latter for embryo culture.

### Barasertib treatment

Barasertib was made as a stock solution of 5 mM in DMSO and stored at –20 °C. Before use, the stock solution was diluted in PZM-3 medium to final concentrations of 3, 5 and 7  $\mu$ M. Control groups were cultured with an identical concentration of DMSO only. Mitotic division rates and mitotic stages of embryo treated with 3, 5 and 7  $\mu$ M inhibitor were examined using stereoscopic microscope and confocal microscopy after culture for 48 h. The chromosome condensation status and spindle phenotypes of embryo treated with 5  $\mu$ M inhibitor were examined using confocal microscopy after culture for 18 and 20 h, respectively.

### Immunostaining and confocal microscopy

The embryos were fixed in 4% paraformaldehyde (PFA) at room temperature for 30 min and permeabilized in 1% Triton X-100 in PBS at room temperature for 12 h. Then, embryos were blocked for 1 h with 1% BSA in PBS and incubated overnight at 4 °C or for 4 h at room temperature with anti-histone H3 (phospho S10) (1:100) or anti- $\alpha$ -tubulin (1:200) FITC-labeled antibodies. The embryos were washed thrice in 0.1% Tween 20 in PBS and incubated with TRITC-labeled goat anti-rabbit IgG (1:100) at room temperature for 1 h. Then, these samples were stained with Hoechst 33,342 (10  $\mu$ g/mL) for 10 min. Finally, they were mounted onto glass slides and examined with a confocal laser scanning microscope (Zeiss LSM700 META, Oberkochen, Germany). In the negative control, the rabbit polyclonal anti-histone H3 (phospho S10) antibody was not added in the embryo.

## Protein extraction and Western blot analysis

A total of 100 embryos at different stages (prophase, prometaphase, meta-anaphase and cytokinesis) were collected after culture for 18, 20, 22 or 24 h, respectively. Samples were lysed in SDS sample buffer and then heated at 100 °C for 10 min. Samples were frozen at −20 °C until use. Total proteins were separated by SDS-PAGE with a 5% stacking gel and a 12% separating gel at 80 V for 20 min and 120 V for 1 h, respectively. Proteins were electrically transferred to a polyvinylidene fluoride (PDVF) membrane (Millipore, Billerica, MA) with a 0.45- $\mu$ m pore for 1 h at 300 mA. The PDVF membrane with  $\beta$ -tubulin or P-H3S10 was blocked by adding 5% BSA or skimmed milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST) for 2 h. After three times washing with TBST, the membrane was incubated with rabbit polyclonal anti-Histone H3 (phospho S10) (diluted 1:1000 in TBST) or rabbit polyclonal anti- $\beta$ -tubulin antibody (diluted 1:3000 in TBST) at 4 °C overnight. After three times washing, the membranes were incubated for 2 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (diluted 1:3000 in TBST; Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was washed three times in TBS for 10 min each. Finally, the specific proteins reacted with chemiluminescence HRP substrate (Millipore, Billerica, MA) and were viewed using the ECL detection system (Amersham International, Buckinghamshire, UK).

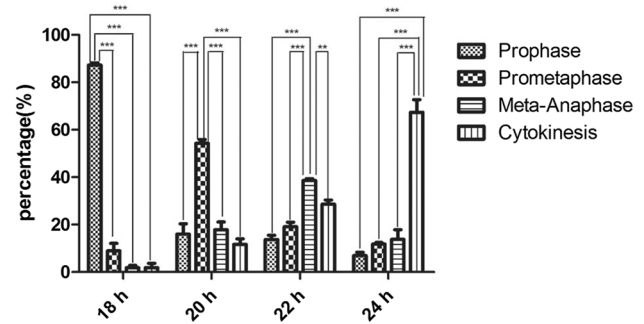
## Statistical analysis

All experiments were performed at least three times. Statistical comparisons were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test. The results were shown as the mean  $\pm$  SEM values. *P* value <0.05 was considered significant.

## Results

### Analysis of cell cycle progression during the first mitotic division in porcine embryo

Given that minimal cell cycle progression data have been reported previously, cell cycle progression of porcine embryos during first mitotic division was examined first in this study. Embryos cultured at different times were collected, and Hoechst 33,342-labeled chromosomes were examined by confocal laser-scanning microscopy. As shown in Fig. 1, most embryos reached the prophase stage, prometaphase stage, meta-anaphase stage and cytokinesis after culturing for 18 h, 20, 22 and 24 h, respectively. The first porcine embryonic mitotic division occurs from 18



**Fig. 1** Analysis of cell cycle progression during the first mitotic division in porcine embryos. Porcine embryo samples were collected at different time point of culture for cell cycle analysis. Most embryos achieved prophase stage, prometaphase stage, meta-anaphase stage and cytokinesis after being cultured for 18, 20, 22 and 24 h, respectively. \*Significant difference ( $P < 0.05$ )

to 24 h in culture, and our subsequent investigations were based on this analysis of cell cycle progression.

### Dynamic distribution of cytoskeleton (microtubule and microfilament) in porcine embryos during the first mitotic division

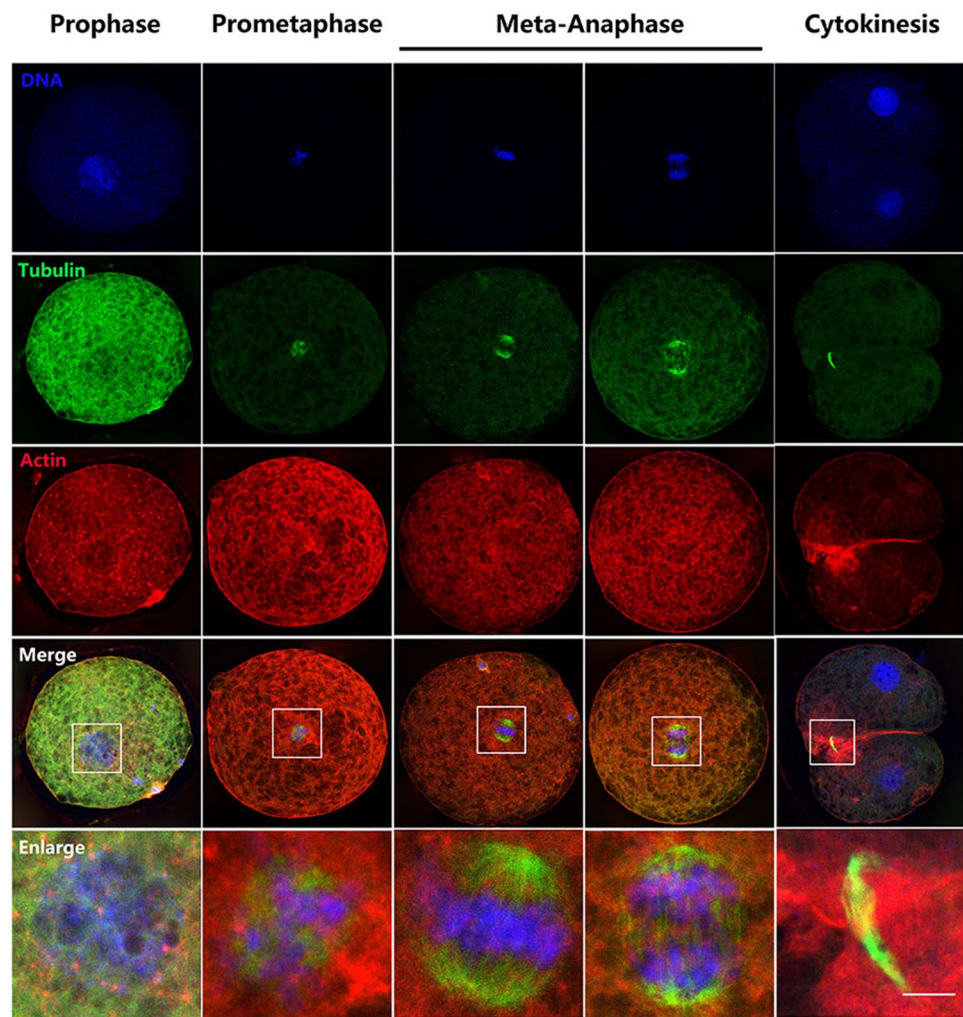
To further determine cell cycle progression during the first mitotic division in porcine embryos, the cytoskeleton (microtubule and microfilament) in porcine embryos was detected by immunofluorescent staining and laser scanning confocal microscopy. As shown in Fig. 2, microtubules were found throughout the cytoplasm at the prophase stage. At the prometaphase stage, microtubules were detected in association with chromatin mass and encompassed the chromatin. Microtubules formed a symmetric and barrel-shaped structure spindle at metaphase stage. During anaphase, microtubules were found in the mitosis spindle. At cytokinesis, microtubules were detected in the mitosis midbody. Microfilaments were observed as a relatively thick and uniform area around the cell cortex and were also distributed throughout the cytoplasm of embryos at different stages during the mitosis. Microfilaments were particularly condensed at the cleavage groove during cytokinesis.

### H3S10ph levels and subcellular localization in porcine embryos during the first mitotic division

To investigate the possible role of H3S10ph in porcine embryos during the first mitotic division, the level of H3S10ph in porcine embryos at different stages of mitotic division was examined using Western blot and immunofluorescent staining. As shown in Fig. 3a, the presence of H3S10ph was detected continuously from prophase to cytokinesis in porcine embryos during the first cleavage.



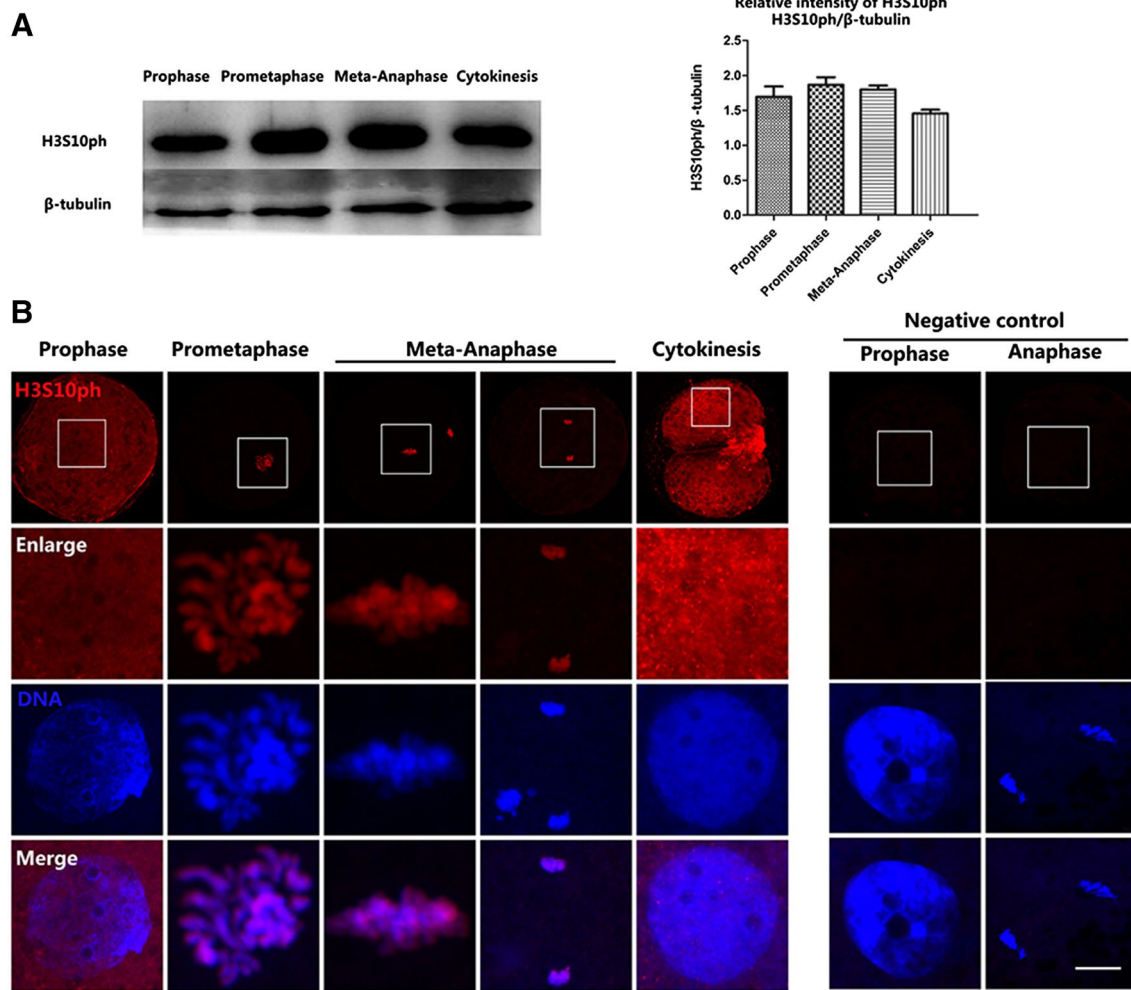
**Fig. 2** Dynamic distribution of microtubules and microfilaments in porcine embryos during the first mitotic division. Microtubules were found throughout the cytoplasm at the prophase stage and in association with the chromatin mass and encompassed the chromatin at the prometaphase stage. At the metaphase stage, microtubules formed a symmetric, *barrel-shaped* structure spindle. Microtubules were detected in the spindle at the anaphase stage. At cytokinesis, microtubules were detected in the mitosis midbody. Microfilaments were observed as a relatively thick uniform area around the cell cortex and were also distributed throughout the cytoplasm of embryos at different stages during the mitosis, especially concentrated on the cleavage furrow during cytokinesis. *Blue* chromatin, *green*  $\alpha$ -tubulin, *red* actin. Scale bar 5  $\mu$ m



H3S10ph levels were maximal during prometaphase and reduced during meta-anaphase and cytokinesis. A dynamic subcellular localization pattern of H3S10ph during the first embryo mitosis is presented in Fig. 3b. H3S10ph was distributed throughout the cytoplasm of embryos during prophase and then concentrated on chromatin during prometaphase, metaphase and anaphase. When embryos progressed to cytokinesis, H3S10ph was distributed throughout the cytoplasm of the embryo. Negative control showed no specific signals. We also performed  $\alpha$ -tubulin and H3S10ph co-staining as shown in Fig. 4, and the results further revealed a similar co-localization pattern for H3S10ph and chromatin during prometaphase, metaphase and anaphase. These temporal and spatial localization patterns suggested that H3S10ph is closely involved in chromosomal dynamics in porcine embryos during the first mitotic division.

#### Barasertib treatment results in first mitotic division failure in porcine embryos

Aurora-B kinase is responsible for histone H3 phosphorylation in *Xenopus* egg cell-free extracts, *Drosophila* and mammalian cells (Jelinkova and Kubelka 2006; Giet and Glover 2001; Murnion et al. 2001; Goto et al. 2002). Thus, a highly selective Aurora-B inhibitor, barasertib, was used in this study to further elucidate the function of H3S10ph in porcine embryos during the first mitotic division. A large proportion of barasertib-treated embryos failed to complete the first mitotic division in a concentration-dependent manner, whereas most of the control embryos successfully developed to the two-cell stage (Fig. 5a). The percentage of embryo mitotic division in the control group was  $80.67 \pm 1.60\%$  ( $n = 189$ ), and the percentage significantly decreased to  $42.20 \pm 2.20\%$  ( $n = 171$ ;  $P < 0.001$ ),



**Fig. 3** H3S10ph levels and subcellular localization in porcine embryos during the first mitotic division. **a** The level of H3S10ph was detected by Western blot. H3S10ph was found in the first mitotic division in porcine embryos. H3S10ph levels were maximal during prometaphase, reduced during meta-anaphase and reached minimum levels during cytokinesis. **b** The subcellular localization of H3S10ph at different stages during the first mitotic division. H3S10ph was dis-

tributed throughout the cytoplasm of embryos during prophase and then concentrated on chromatin during prometaphase, metaphase and anaphase. However, when embryos progressed to cytokinesis, H3S10ph was distributed throughout the cytoplasm of embryo. Negative control showed no specific signals. Red H3S10ph, blue chromatin, Scale bar 5  $\mu$ m

$14.24 \pm 1.71\%$  ( $n=173$ ;  $P<0.001$ ), and  $2.07 \pm 0.50\%$  ( $n=198$ ;  $P<0.001$ ) when treated with 3, 5 and 7  $\mu$ M of barasertib, respectively (Fig. 5b). These results indicate that the Aurora-B inhibitor caused the failure of the first mitotic division in porcine embryos.

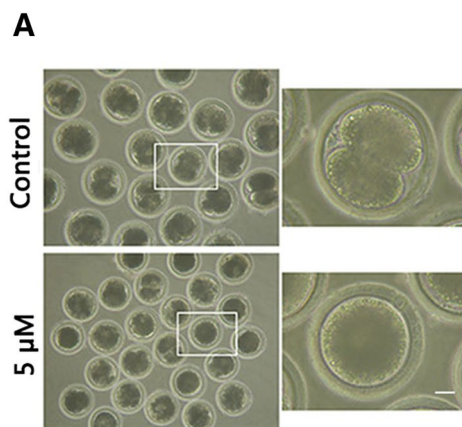
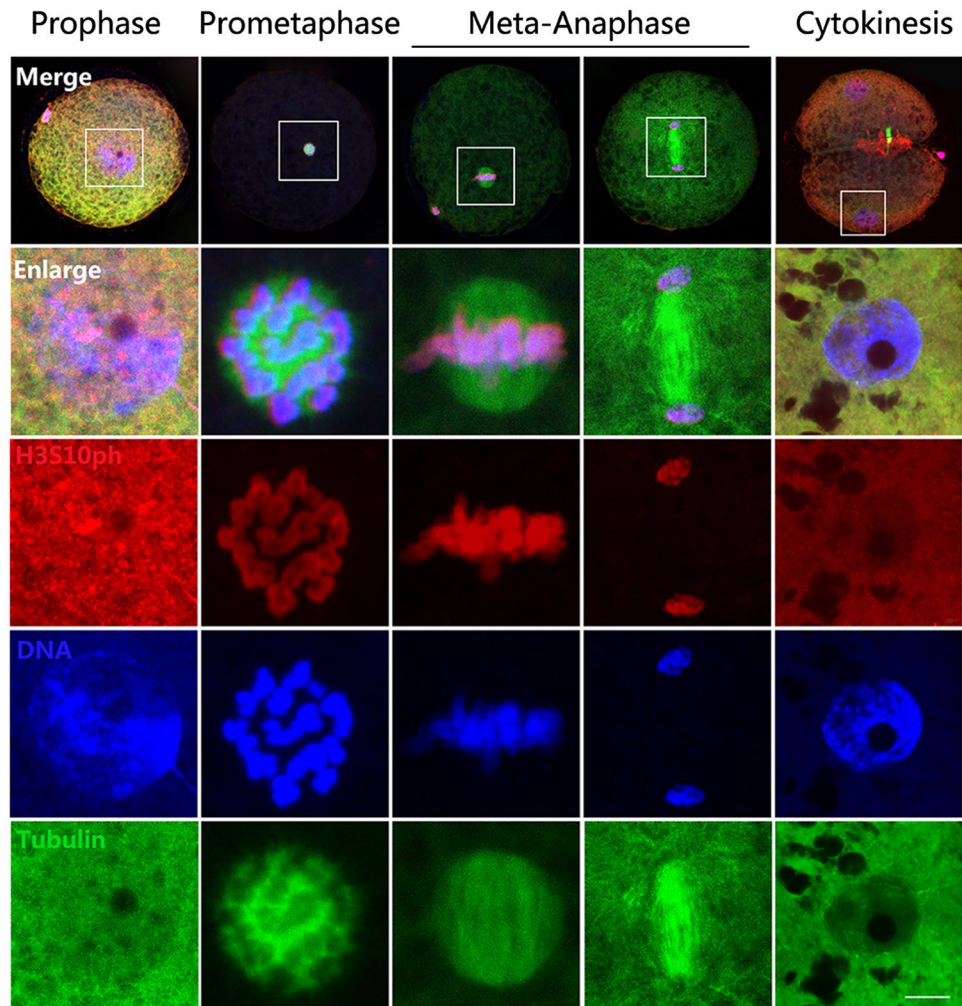
**Barasertib treatment results in disruption of cell cycle progression during the first mitotic division in porcine embryos**

To further examine the roles of H3S10ph in regulating the cell cycle in embryos, we determined the proportions of porcine embryos arrested at different mitotic stages with barasertib. The mitotic stages of embryos were assessed

through analysis of Hoechst 33342-labeled chromosomes under a confocal laser-scanning microscope. As shown in Fig. 6, after 5  $\mu$ M barasertib treatment for 48 h, most control embryos completed the first mitotic division. However, the percentage of embryos that completed the first mitotic division was sharply reduced after barasertib treatment, whereas the proportion of embryos that were arrested at the prophase stage was increased significantly after barasertib treatment in an inhibitor concentration-dependent manner (Fig. 6a). In the control group, the percentage of embryo that succeeded in finishing the first mitotic division was  $85.93 \pm 4.27\%$  ( $n=182$ ). However, the percentage was significantly reduced to  $43.07 \pm 4.60\%$  ( $n=173$ ;  $P<0.001$ ),  $13.94 \pm 1.71\%$  ( $n=177$ ;  $P<0.001$ ),

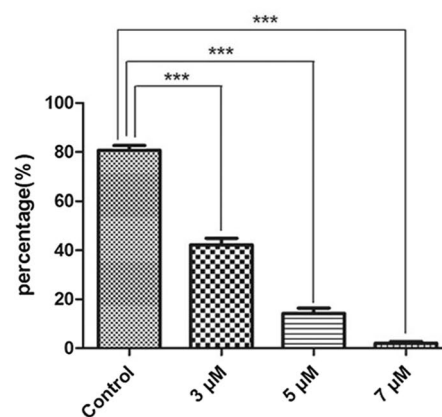


**Fig. 4** Immunofluorescent staining for co-localization of H3S10ph and  $\alpha$ -tubulin during the first mitotic division in porcine embryos. The results revealed a similar localization pattern between H3S10ph and chromatin during prometaphase, metaphase and anaphase. Red H3S10ph, Blue chromatin, green  $\alpha$ -tubulin, Scale bar 5  $\mu$ m

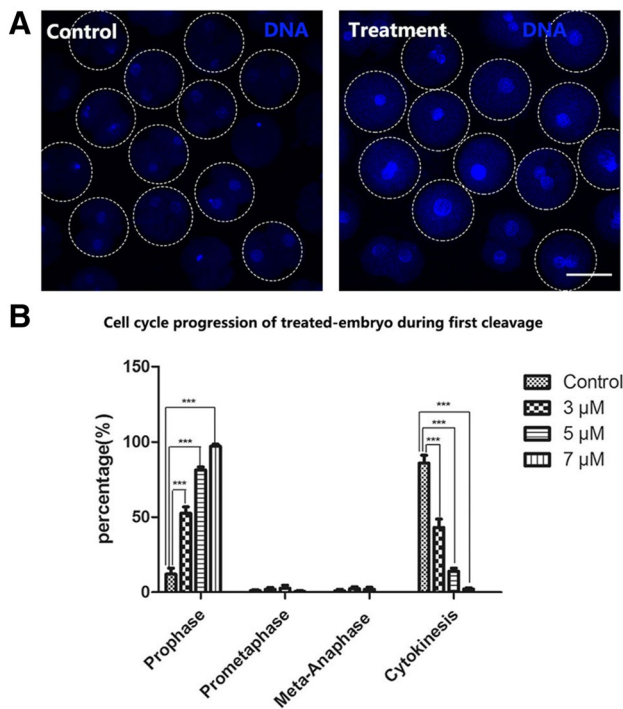


**Fig. 5** Barasertib treatment results in first mitotic division failure in porcine embryos. **a** Representative image of the first mitotic division in porcine embryos in the presence/absence of barasertib. After 48 h in culture, most of the control embryos successfully developed to the two-cell stage, whereas a large proportion of the barasertib-treated embryos failed to complete the first mitotic division. Scale

**B** Rate of first embro cleavage



bar, 20  $\mu$ m. **b** Barasertib treatment resulted in a decreased mitotic division proportion in a concentration-dependent manner. Compared with the control, the first mitotic division proportion significantly decreased after 3, 5 and 7  $\mu$ M barasertib treatment. \*Significant difference ( $P < 0.05$ )



**Fig. 6** Barasertib treatment disrupts cell cycle progression during the first mitotic division in porcine embryos. **a** Representative image of the first mitotic division in porcine embryos in the presence/absence of barasertib specifically showing the chromosome dynamics during mitosis. The mitotic stages of embryos were assessed via Hoechst 33342-labeled chromosomes under a confocal laser-scanning microscope. After 5  $\mu\text{M}$  barasertib treatment for 48 h, most control embryos completed the first mitotic division. However, the percentage of embryos that completes the first mitotic division was sharply reduced after barasertib treatment, whereas the proportion of embryos that were arrested at the prophase stage was increased significantly after Aurora-B inhibition. The dotted white line circle in the control group shows the embryos that succeeded in mitotic division, and the embryos that were arrested at the prophase stage in the treatment group are noted. Blue DNA, Scale bar 80  $\mu\text{m}$ . **b** The embryos were blocked at the prophase stage and could not proceed through the mitotic cell cycle after Aurora-B inhibition. Compared with the control, the percentage of embryos that reached the mitotic division stage was sharply reduced, whereas the proportion of embryos arrested at the prophase stage was significantly increased in an inhibitor concentration-dependent manner after 3, 5 and 7  $\mu\text{M}$  of barasertib treatment. \*Significant difference ( $P < 0.05$ )

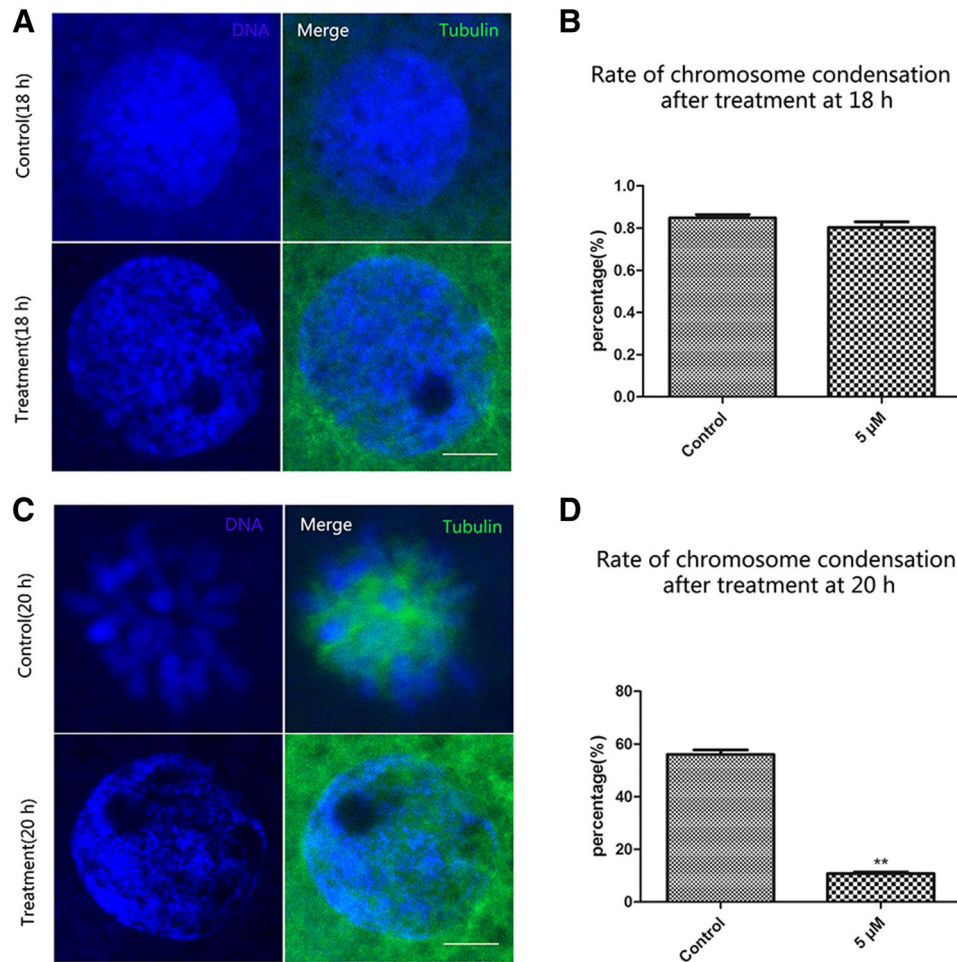
and  $2.19 \pm 0.57\%$  ( $n = 193$ ;  $P < 0.001$ ) when treated with 3, 5 and 7  $\mu\text{M}$  of barasertib, respectively. Furthermore, the percentage of control embryos arrested at the prophase stage was  $12.04 \pm 3.40\%$  ( $n = 182$ ), whereas the levels in the treatment groups were significantly increased to  $52.53 \pm 3.52\%$  ( $n = 173$ ,  $P < 0.001$ ),  $81.42 \pm 1.60\%$  ( $n = 177$ ) and  $97.23 \pm 1.06\%$  ( $n = 193$ ;  $P < 0.001$ ) after 3, 5 and 7  $\mu\text{M}$  of barasertib treatment, respectively (Fig. 6b). These data indicate that porcine embryo mitotic division progression was disrupted, and embryos were blocked at the prophase stage upon Aurora-B inhibition.

### Barasertib treatment results in defects in chromosome condensation during porcine embryo first mitotic division

As the embryos were blocked at prophase stage after treatment with the Aurora-B inhibitor barasertib, we sought to further clarify the reasons for porcine embryo failure to progress to the prometaphase stage. Chromosome condensation in the embryos was examined after 5  $\mu\text{M}$  barasertib inhibition for 18 and 20 h, when most embryos should achieve the prophase and prometaphase stage, respectively. As shown in Fig. 7, there was no obvious difference in chromosome condensation morphology. The nuclear membrane remains intact in the control and treatment groups for 18 h (Fig. 7a). However, the percentage of embryos with condensed chromosomes that reached prometaphase was sharply reduced after treatment with barasertib for 20 h, whereas the proportion of embryos arrested at the prophase stage increased significantly after Aurora B inhibition (Fig. 7c). In the control group,  $84.86 \pm 1.33\%$  ( $n = 123$ ) of embryos exhibited normal chromosome condensation morphology compared with  $80.41 \pm 2.17\%$  ( $n = 180$ ) in the 5  $\mu\text{M}$  barasertib-treated embryos after culture for 18 h. ( $P > 0.05$ , Fig. 7b). In the control group,  $56.04 \pm 1.42\%$  ( $n = 102$ ) of embryos exhibited normal chromosome condensation morphology compared with  $10.80 \pm 0.47\%$  ( $n = 121$ ) in the 5  $\mu\text{M}$  barasertib-treated embryos ( $P < 0.001$ , Fig. 7d) after culture for 20 h. These results suggest that Aurora-B inhibition results in defects in chromosome condensation at the prometaphase stage and disrupts cell cycle progression during the first mitotic division porcine embryos.

### Barasertib treatment results in reduction in H3S10ph during the first mitotic division in porcine embryos

To further explore the reasons for the porcine embryos' failure to undergo chromosome condensation after barasertib treatment and to assess the correlation among Aurora-B activity, H3S10ph and chromosome condensation during the first mitotic division of pig embryos, H3S10 phosphorylation in embryos was examined after 5  $\mu\text{M}$  barasertib treatment for 18 and 20 h. As shown in Fig. 8, barasertib treatment significantly eliminated the phosphorylation of H3S10 in porcine embryos at 18 and 20 h of the culture ( $P < 0.001$ ). The results indicate that the defect in chromosome condensation is accompanied with the reduction of H3S10ph, which is eliminated by Aurora-B inhibition. This finding indicates that H3S10ph is essential for chromosome condensation during the first mitotic division in pig embryos.



**Fig. 7** Barasertib treatment results in defects in chromosome condensation during mitotic division in porcine embryos. **a** and **c** Representative images of chromosome condensation morphology in porcine embryos in the presence/absence of barasertib treatment for 18 and 20 h, respectively. Blue chromatin, green  $\alpha$ -tubulin, Scale bar 5  $\mu$ m. **b** and **d** Proportion of embryos with chromosome condensation morphology after barasertib treatment. There was no obvious difference in chromosome condensation between the control and treatment

groups when cultured for 18 h. However, compared with the control, the percentage of embryos that reached the prometaphase stage was sharply reduced, whereas proportion of embryos arrested at the prophase stage was significantly increased after 5  $\mu$ M barasertib treatment for 20 h. These results indicate that Aurora-B inhibition results in defects in chromosome condensation at the prometaphase stage and disruption of cell cycle progression during the first mitotic division in porcine embryos. \*Significant difference ( $P < 0.05$ )

## Discussion

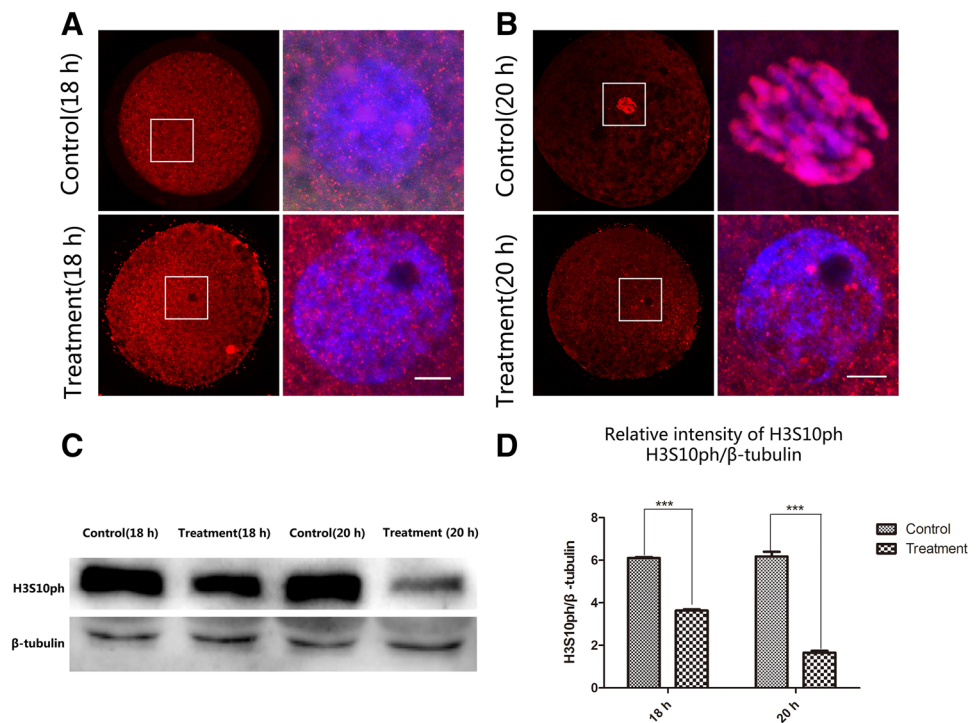
The function of H3S10ph was studied extensively in both somatic cells and germ cells, but little is known about its role in porcine embryos during first mitosis division. In this study, we investigated the subcellular localization and possible functions of H3S10ph in porcine embryos during the first mitotic division. Our results indicate that H3S10ph contributed to the regulation of chromosome condensation and cell cycle progression in porcine embryos during the first mitosis division.

Data have not been reported about the exact time points of cell cycle progression during the first mitotic division in porcine embryos. Thus, we first assessed the actual stage at different time points by immunocytochemistry and laser

scanning confocal microscopy. The results show that the first mitotic division in porcine embryos occurs from 18 to 24 h in culture. To further examine the cell cycle progression of porcine embryos during the first mitotic division, the distribution of cytoskeleton (microtubule and microfilament) at different cell cycle stages was examined. The results show that both microtubules and microfilaments exhibit dynamic localization during the first mitotic division of porcine embryos. Kim et al. (1997) reported that microtubule and microfilament assembly inhibited by nocodazole and cytochalasin B, respectively, results in the failure of cell division during fertilization in pigs. Successful fulfillment of mitotic division requires spatial coordination of dynamic chromosomal and spindle events (Paul 2000), such as chromosome condensation and alignment, bipolar



**Fig. 8** Barasertib treatment reduces H3S10ph in the first mitotic division in porcine embryos. **a, b** Representative images of porcine embryo H3S10ph subcellular localization in the presence/absence of barasertib when cultured for 18 h and 20 h, respectively. *Red* H3S10ph, *blue* chromatin, *Scale bar* 5  $\mu$ m. **c, d** H3S10ph was detected by Western blotting between control and barasertib treatment groups for 18 and 20 h, and barasertib treatment significantly caused the reduction of H3S10ph in porcine embryo mitosis for 18 and 20 h ( $P < 0.001$ ). \*Significant difference ( $P < 0.05$ )



spindle formation and kinetochore–microtubule interaction (Lampson et al. 2004).

To determine the potential role of H3S10ph during the first mitotic division in porcine embryos, we first examined the level and localization of H3S10ph. Western blotting results show that H3S10ph was continuously present during the entire first mitotic division and was maximal during prometaphase, dephosphorylated slightly during meta-anaphase and cytokinesis (Fig. 3a). Laser scanning confocal microscopy also revealed dynamic localization of H3S10ph distributed throughout the cytoplasm of embryos during prophase and cytokinesis, concentrated on chromosomes during prometaphase, metaphase and anaphase stage. Difference was found from configuration of this pig embryo's phosphorylation to that of some mammalian somatic cells (HeLa cells, SKN cells et.al) and *Tetrahymena* (Wei and Allis 1998), where phosphorylation on Ser-10 of H3S10 induced from G2 phase to metaphase and dephosphorylation of large H3S10 was completed in telophase from the beginning of anaphase showing a strong correlation between Ser10 phosphorylation and chromosome condensation during cell division (Hendzel et al. 1997; Prigent and Dimitrov 2003; Van Hooser et al. 1998). Our data exhibited the dynamic distribution pattern of H3S10ph during the first mitotic division in porcine embryos was similar to a previous report in mouse wherein the H3S10ph signal persisted until metaphase, anaphase and telophase, and a strong H3S10ph signal also emerged in formation of chromosomes during the first mitosis in fertilized mouse

embryos (Teperek-Tkacz et al. 2010). Our results expressed that H3S10ph was concentrated on chromatin during prometaphase, metaphase and anaphase, and distributed throughout the cytoplasm during prophase and cytokinesis during the first mitosis in porcine embryos. Our localization pattern suggests that H3S10ph is intimately related in porcine embryo's chromosomal dynamics in the period of first mitotic division.

Moreover, a weak relationship unveiled between phosphorylation at Ser10 of histone H3 and chromosome dynamics in *S. cerevisiae* (Hsu et al. 2000) and *Drosophila* (Adams et al. 2001) recommends that only histone H3 phosphorylation cannot be liable for chromosome condensation. Previous reports have demonstrated that H3S10ph is closely associated with mitotic chromosome condensation in many organisms (Hendzel et al. 1997; Nowak and Corces 2004; Strahl and Allis 2000). However, the role of H3S10ph in the progression of mitosis and condensation of chromosomes in porcine embryo cells is unclear. Aurora-B is required for histone H3 phosphorylation on Ser-10 in *C. elegans*, *Drosophila* and *Xenopus* egg cell-free extracts (Giet and Glover 2001, Hsu et al. 2000; Murnion et al. 2001; Adams et al. 2001), and similar conclusions were obtained in Swiss 3T3 cells (Goto et al. 2002). Therefore, in the present study, a highly selective Aurora-B inhibitor barasertib (Mortlock et al. 2007) was used to further explore the role of H3S10ph in porcine embryos during the first mitotic division. Teperek-Tkacz et al. (2010) observed that treatment

with ZM447439 actually halted at the G2 of the second cell cycle in most of the two-cell embryos. We also found that embryos failed to complete the first mitotic division after barasertib treatment (Fig. 5), more importantly, cell-cycle examination further indicated that most of the barasertib-treated embryos were blocked at the prophase stage. The finding suggests that H3S10ph inhibition leads to a failure of mitotic division during the first mitotic division of pig embryos.

To further explore the reasons for the porcine embryos' failure to progress to the prometaphase stage, chromosome condensation and H3S10ph were examined after of barasertib treatment for 18 or 20 h, when most embryos were in the prometaphase to prophase stage. Barasertib treatment lead to chromosome condensation defects, and H3S10ph levels significantly decreased. These results indicate that the defect in chromosome condensation is accompanied by a reduction in H3S10ph. Interestingly, our results were different from a published study in mouse in which the suppression of Aurora B activity with ZM447439 resulted in a decrease in H3S10ph and severe abnormalcy in the spindle building and chromatid sequestration during the first mitotic division of fertilized embryos (Teperek-Tkacz et al. 2010). Our previous study reported that reduction of H3S10ph after barasertib treatment did not result in the defect of chromosome condensation during porcine oocyte meiotic maturation (Ju et al. 2016) potentially due to different mechanisms of chromosome condensation between oocytes meiosis and embryos mitosis. In line with our results, H3S10 phosphorylation by Aurora B is required for chromosome condensation during mitosis in *Tetrahymena*, *Drosophila* and *C. elegans* (Wei and Allis 1998; Giet and Glover 2001, Hsu et al. 2000). This study manifests that H3S10ph is essential for chromosome condensation in porcine embryos during the first mitotic division.

At present, the relationship between H3S10ph and chromosome condensation during mitosis has been explained according to two different models. One model summarizes that phosphorylation on Ser-10 of H3 tail significantly interferes histone H3-DNA interactions (Sauvé et al. 1999). Another model is based on the idea that the condensation factors (such as SMC proteins and topoisomerase II) are enrolled to the chromosomes through direct interactions with phosphorylated histone H3 tail (de la Barre et al. 2000; Kimura and Hirano 2000). Our findings require further studies to provide advanced insights into the specific mechanisms that regulate chromosome condensation during porcine embryos mitosis.

In summary, the above results indicate that H3S10ph is involved in the first mitotic division in porcine embryos through its regulatory function on chromosome condensation, which further affects the cell cycle and first mitotic division in porcine embryos. Phosphorylation of histone

H3 on Ser-10 is essential for chromosome condensation in porcine embryos during the first mitotic division.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The animals used in this study and their care were according to the guidelines of Animal Research Institute Committee which is prescribed by Nanjing Agricultural University, China. The workers who executed the slaughtering complied with the pig slaughtering regulations (State Council of the People's Republic of China, No. 666).

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