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CHD1 Regulates Deposition of Histone Variant H3.3 During Bovine Early Embryonic Development[1]

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ABSTRACT

The CHD family of proteins is characterized by the presence of chromodomains and SNF2-related helicase/ATPase domains, which alter gene expression by modification of chromatin structure. *Chd1*-null embryos arrest at the peri-implantation stage in mice. However, the functional role of CHD1 during preimplantation development remains unclear, given maternal-derived CHD1 may mask the essential role of CHD1 during this stage in traditional knockout models. The objective of this study was to characterize *CHD1* expression and elucidate its functional role in preimplantation development using the bovine model. *CHD1* mRNA was elevated after meiotic maturation and remained increased through the 16-cell stage, followed by a sharp decrease at morula to blastocyst stage. Similarly, immunoblot analysis indicated CHD1 protein level is increased after maturation, maintained at high level after fertilization and declined sharply afterwards. *CHD1* mRNA level was partially decreased in response to alpha-amanitin (RNA polymerase II inhibitor) treatment, suggesting that *CHD1* mRNA in eight-cell embryos is of both maternal and zygotic origin. Results of siRNA-mediated silencing of *CHD1* in bovine early embryos demonstrated that the percentages of embryos developing to the 8- to 16-cell and blastocyst stages were both significantly reduced. However, expression of *NANOG* (inner cell mass marker) and *CDX2* (trophectoderm marker) were not affected in *CHD1* knockdown blastocysts. In addition, we found that histone variant H3.3 immunostaining is altered in *CHD1* knockdown embryos. Knockdown of *H3.3* using siRNA resulted in a similar phenotype to *CHD1*-ablated embryos. Collectively, our results demonstrate that CHD1 is required for bovine early development, and suggest that CHD1 may regulate H3.3 deposition during this period.

CHD1, *cattle, embryo, epigenetics, H3.3, preimplantation*

INTRODUCTION

Female infertility poses a great challenge for both animal agriculture and human medicine. Reproductive efficiency is a major limiting factor that impacts productivity and profitability for the dairy industry. Recent data show that the conception rate to first breeding is only approximately 32% in high-producing cows [1–3]. In a recent review, Sartori et al. estimated that 37% of embryo deaths occur during the first week after fertilization, indicating that early embryonic loss is a major contributing factor to infertility in dairy cows [1]. Meanwhile, mounting evidence shows that children conceived by assisted reproductive technologies (ARTs) are at higher risk of preterm delivery, low birth weight, and genomic imprinting disorders, which are attributed to the exposure to manipulation during ARTs [4]. Therefore, fundamental understanding of the molecular regulation of early embryos is critical for improved ARTs practice and development of novel strategies toward improving cow fertility.

CHD1 belongs to CHD (chromodomain DNA binding protein) family of proteins, which are characterized by the presence of chromodomains (chromatin organization modifier) and SNF2-related helicase/ATPase domains. CHD1 is not only implicated in transcription-related chromatin remodeling, but is required to maintain a specific chromatin configuration across the genome [5, 6]. For instance, the tandem chromodomain of human CHD was shown to recognize and bind specifically to trimethylation of histone H3 lysine 4 (H3K4me3; an epigenetic modification associated with active transcription), and thus moderates subsequent recruitment of factors responsible for posttranscriptional initiation and pre-messenger-RNA splicing [6, 7]. In *Drosophila*, maternal Chd1 is required for the incorporation of histone variant H3.3 into the paternal pronucleus immediately after fertilization [8]. Eventually, eggs produced by crossing *Chd1*-mutant female *Drosophila* and wild-type males die before hatching [8]. RNAi-mediated depletion of *Chd1* in mouse embryonic stem cells (ESCs) results in a loss of pluripotency, suggesting an important role of CHD1 in mammalian early development in vivo [9]. One recent study shows that *Chd1* knockout embryos undergo developmental arrest at Embryonic Day (E) 6.5 due to a failure to maintain epiblast development [10]. However, the functional role of CHD1 in preimplantation development remains elusive, as maternal CHD1 may mask its role during this stage in traditional knockout models. Therefore, we hypothesize that CHD1 is required for early embryo development in cattle.

To test the hypothesis, we characterized CHD1 expression in bovine early embryos and used our unique and robust RNAi

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system in early embryos, whereby small interfering RNAs (siRNA) are delivered into embryos by microinjection immediately after fertilization. Our results show that *CHD1* in bovine early embryos is not only transcribed from maternal genome, but also from zygotic genome. Deletion of CHD1 led to reduced developmental rates to the blastocyst stage and reduced H3.3 signal relative to controls. We also demonstrate that siRNA-mediated silencing of H3.3 results in similar phenotypes with *CHD1* ablation in bovine embryos. Our work provides evidence supporting a crucial role of CHD1 in promoting bovine early embryonic development. Furthermore, results suggest a functional role for CHD1 in bovine early development that is likely mediated by regulation of H3.3 deposition.

MATERIALS AND METHODS

Materials

Chemicals and reagents were purchased from Sigma unless otherwise indicated.

In Vitro Maturation, In Vitro Fertilization, and In Vitro Embryo Culture

Bovine ovaries were collected from a local slaughterhouse. After transferring back to the laboratory, cumulus-oocyte complexes (COCs) were aspirated from follicles 2–6 mm in diameter. Those COCs with intact cumulus cells were selected for use in subsequent experiments. In vitro maturation medium was Medium 199 with Earle salts and supplemented with 1 μg/ml estradiol-17β, 1 IU/ml FSH, 5 IU/ml LH (Sioux Biochemical), and 10% fetal bovine serum (Gibco-BRL).

After 22–24 h, COCs (50 COCs/well in a 4-well plate) were incubated with spermatozoa (1×10^6 sperm/ml), which were purified from frozen-thawed semen by centrifuging through a Percoll gradient (30%–60%–90%) [11, 12]. In vitro fertilization (IVF) was performed at 38.5°C under 5% CO_2 in humidified air for 16–18 h. To remove cumulus cells, putative zygotes were vortexed for 5 min. After washing, zygotes were cultured in potassium simplex optimization medium (KSOM; MR-121-D; Millipore) supplemented with 0.3% bovine serum albumin (BSA) at 38.5°C under 5% CO_2 in humidified air. At 72 h postinsemination (hpi), 8- to 16-cell embryos were transferred to fresh KSOM containing 0.3% BSA and 10% fetal bovine serum until Day 7.

To select oocytes by Brilliant Cresyl Blue (BCB) staining, COCs were incubated with or without 26 μM BCB in buffered saline that contained 0.4% BSA for 90 min [13]. After two washes, COCs treated with BCB were divided into two groups based on the cytoplasm color: oocytes with blue color cytoplasm (BCB+) and oocytes with no blue cytoplasm (BCB−).

Examination of CHD1 Expression in Bovine Oocytes and Early Embryos

Quantitative PCR (qPCR) analysis of oocytes/embryos was performed as published previously [11, 14, 15]. Random hexamers were used for reverse transcription. Oocytes in germinal vesicle and metaphase II (24 h after initiation of maturation) stage were collected. Embryos were collected at the following stages/hpi: zygotes, 20 hpi; 2 cell, 33 hpi; 4 cell, 44 hpi; 8 cell, 52 hpi; 16 cell, 72 hpi; and morula and blastocysts at Days 5 and 7 (Day 0 refers to the day of IVF; n = 10 oocytes/embryos per time point; n = 4 replicates). The primer sequences used were: *CHD1* (NM_001192048.1)-forward: TGGG CTTCTCAAATGAACGC, reverse: GTTTGGTCTGGGGATGCATC; *H3F3A* (NM_001014389.2)-forward: TTTTTCCATGGGGTCAAAAG, reverse: TGGAAAAACTGCCAATACCTG; *H3F3B* (NM_001242571.2)-forward: GTGGTGGGGAGTGTTGTCTT, reverse: AACGCGCAAAGC ATTTTACT; *CXCL6* [16]; *NANOG* [11]; *CDX2* [11]; *GAPDH* [16]; *ACTB* [16]; *RPL15* [16]; cyclophilin-A [16]; *RPL19* [16]; *RPS18* [11]. To determine if *CHD1* mRNA in early embryos was of maternal or zygotic origin, zygotes were treated with or without 50 μg/ml α-amanitin (RNA polymerase II inhibitor), and eight-cell embryos were collected (n = 10 embryos/group, n = 4 replicates).

RNAi in Bovine Preimplantation Embryos

RNAi in bovine early embryos was performed as we described previously [12, 17–19]. For all siRNAs, BLAST analysis was performed to avoid nonspecific targets. The antisense and sense oligo template sequences used in this study were: *CHD1* siRNA 1-antisense: AAGATTGGCAGATGTCTG GATCCTGTCTC, sense: AAATCCAGACATCTGCCAATCCCTGTCTC; *CHD1* siRNA 2-antisense: AAGCAACTGTCAATGTTAGCTCCTGTCTC, sense: AAAGCTAACATTGACAGTTGCCCTGTCTC; *H3F3A* siRNA 1-antisense: AATTAGACGTTATCAGAAGTCCCTGTCTC, sense: AA GACTTCTGATAACGTCTAACCTGTCTC; *H3F3A* siRNA 2-antisense: AAGTGAGGCCTATCTGGTTGGCCTGTCTC, sense: AACCAACCAGAT AGGCCTCACCCTGTCTC; *H3F3B* siRNA 1-antisense: AATCCGTCGT TACCAGAAATCCCTGTCTC, sense: AAGATTTCTGGTAACGACG GACCTGTCTC; *H3F3B* siRNA 2-antisense: AACCGACTTGAGGTTCCA GAGCCTGTCTC, sense: AACTCTGGAACCTCAAGTCGGCCTGTCTC. Each individual siRNA was validated in silencing endogenous target genes in early embryos. Then, a mixture of two effective siRNAs/gene (25 μM, 20 pl) was microinjected into zygotes (16–18 hpi). Four-cell embryos (42–44 hpi) were used for qPCR analysis to verify RNA knockdown efficiency (n = 10 embryos/pool; n = 3 replicates). As controls, zygotes were either uninjected or injected with a universal negative control siRNA (25 μM, no. 1; Ambion).

To test the knockdown efficiency of siRNAs at the protein level, Western blotting (CHD1) or immunofluorescence (H3.3) was performed in early embryos (Western blotting: n = 20–40 embryos/treatment, n = 6 replicates; immunostaining: n = 10 embryos/treatment, n = 4 replicates). To quantify the influence of *CHD1* or *H3.3* knockdown on the developmental potential of bovine early embryos, total cleavage rate (48 hpi) and the proportion of embryos developing to the 8- to 16-cell (72 hpi) and blastocyst (Day 7) stages were determined (n = 25–30 embryos/treatment, n = 6 replicates).

Immunofluorescence

Embryos were fixed in 4% paraformaldehyde/PBS for 30 min, permeabilized in 0.25% Triton X-100/PBS for 25 min, and blocked in 5% BSA/PBS for 1 h at room temperature. Then, embryos were treated with primary antibody (mouse anti-H3.3, 1:200 in blocking buffer [Abnova]; rabbit anti-H3K4me3, 1:200 [Cell Signaling]) overnight at 4°C. Following three washes, incubation with secondary antibody (anti-mouse IgG-Alexa Fluor 488, anti-rabbit IgG-Alexa Fluor 546 [Life Technologies], 1:1000 in PBS containing 1% BSA and 0.1% Tween 20) was performed for 1 h. Embryos were loaded onto slides with ProLong Gold Antifade Reagent with 4′,6-diamidino-2-phenylindole (Life Technologies). In the negative control group, all the above procedures were performed, except that primary antibodies were not used. Imaging of embryos was performed with epifluorescence microscopy under 200-fold magnification.

Western Blot Analysis

Oocytes/embryos (n = 40 oocytes/embryos per pool) were collected in RIPA buffer. After treatment with sample buffer (Laemmli buffer), lysates of embryos were loaded into precast gels (4%–20%; Bio-Rad). The proteins were separated at 150 V for 70–90 min. Then, membrane transfer was performed at 95 V for 65 min at 4°C. After transfer, membranes were incubated for 1 h in blocking buffer (Tris-buffered saline pH 7.4 [TBS] with 0.1% [v/v] Tween 20 and 5% [w/v] BSA at room temperature). Afterwards, membranes were incubated with the primary antibodies, anti-CHD1 (Millipore) at 1:1000 dilution, anti-actin (Millipore) at 1:5000 dilution in blocking buffer on a rotating shaker at 4°C overnight. After washing, membrane was incubated with horseradish peroxidase-conjugated anti-mouse-IgG (Thermo Scientific) secondary antibody at 1:5000 in blocking buffer for 1 h at room temperature. Immunoreactive proteins were detected using SuperSignal West Dura Chemiluminescent Substrate (Thermo Scientific), and images obtained with myECL Imager (Thermo Scientific).

Statistical Analysis

All data were analyzed by using one-way ANOVA with SAS 9.4 (SAS Institute). All percentage data were arc-sin transformed prior to data analysis, and data are presented as untransformed. Differences among means of different groups were compared using Fisher protected least significant difference test. The intensity of fluorescence was analyzed using ImageJ, as described previously [20]. In brief, nuclear area was traced and fluorescence intensity was measured. The same outlined region was moved to the cytoplasm in the same cell, and background fluorescence intensity was obtained. The specific signal was generated by subtracting cytoplasmic intensity from nuclear intensity. The intensity values were then normalized to the relative channels in control embryos.

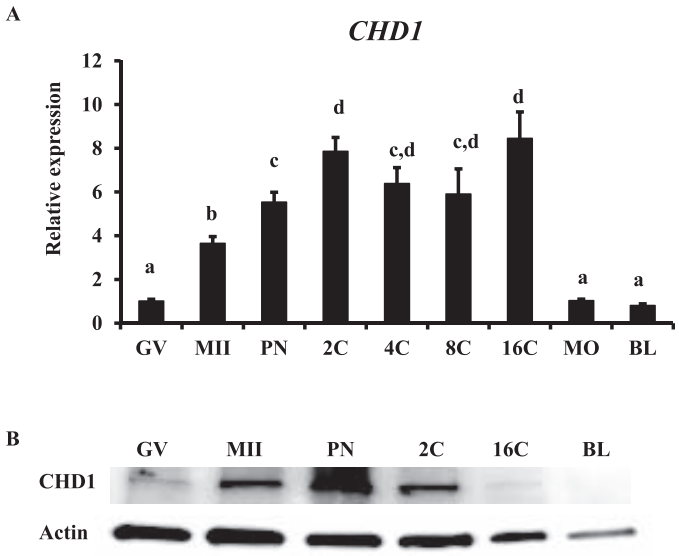

FIG. 1. Dynamic expression profile of CHD1 in bovine early embryos. **A**) qPCR analysis was performed in oocytes and embryos at the following stages: germinal vesicle (GV); metaphase II (MII); zygote (PN); 2-cell (2C); 4-cell (4C); 8-cell (8C); 16-cell (16C); morula (MO); and blastocyst (BL) stages (10 oocytes/embryos per pool, n = 4). **B**) Western blot assay for CHD1 (20–40 oocytes/embryos per pool, n = 3). Data were normalized to endogenous control (RPS18) and are shown as mean ± SEM. Different superscripts across timepoints indicate significant differences ($P < 0.05$).

RESULTS

Dynamic Expression Profile of CHD1 *During Bovine Early Development*

As seen in Figure 1A, qPCR results indicate that *CHD1* mRNA is elevated after meiotic maturation and remains increased through the 16-cell stage, followed by a sharp decrease during the morula-blastocyst transition. Similarly, Western blot analysis using two different sources of antibodies against CHD1 indicated that the level increases after maturation, is maintained at a high level through the 2- to 16-cell stage, and declines sharply thereafter (Fig. 1B). This expression pattern of *CHD1* resembles that of maternally expressed genes. Thus, to determine if *CHD1* mRNA in early embryos is of maternal origin, zygotes were treated with α-amanitin (RNA polymerase II inhibitor). As seen in Figure 2A, qPCR analysis showed that *CXCL6* expression (known zygotic expressed gene) was almost completely suppressed by α-amanitin treatment, indicating the efficacy of α-amanitin in inhibiting transcriptional activity. However, *CHD1* mRNA level only partially decreased in response to α-amanitin treatment (Fig. 2B), suggesting that *CHD1* mRNA is of both maternal and zygotic origin.

CHD1 Is Associated with Developmental Competence of Early Embryos

Initially, to test the relationship between *CHD1* transcript abundance and oocyte quality, qPCR analysis was conducted on oocytes subjected to BCB staining, a well-established model of oocyte quality linked to subsequent embryo developmental potential [21, 22]. As shown in Supplemental Figure S1 (Supplemental Data are available online at www.biolreprod.org), *CHD1* mRNA was lower in oocytes negative for BCB staining (indicative of reduced oocyte quality) than oocytes that had completed the growth phase and were BCB+, suggesting that maternal *CHD1* levels may influence embryogenesis.

Thus, to clarify the functional role of *CHD1* in bovine early embryonic development, siRNAs targeting *CHD1* were produced, and validated procedures for siRNA-mediated ablation of endogenous *CHD1* expression were performed. Microinjection of *CHD1* siRNA cocktail greatly reduced endogenous *CHD1* mRNA by >90% in bovine embryos collected at 42–44 hpi compared to either uninjected control or negative control siRNA-injected embryos (Fig. 3A and Supplemental Fig. S2). Meanwhile, expression of selected genes (housekeeping genes or maternally expressed gene) was not affected by *CHD1* siRNA injection (Supplemental Fig. S3), demonstrating its targeting specificity. A corresponding reduction in CHD1 protein in *CHD1* siRNA-injected embryos was demonstrated by Western blot analysis against CHD1 (Fig. 3B).

To determine the effect of *CHD1* deletion on embryo developmental potential, in vitro development was monitored. Results revealed that the total cleavage rate was not influenced in *CHD1*-ablated embryos relative to controls (data not

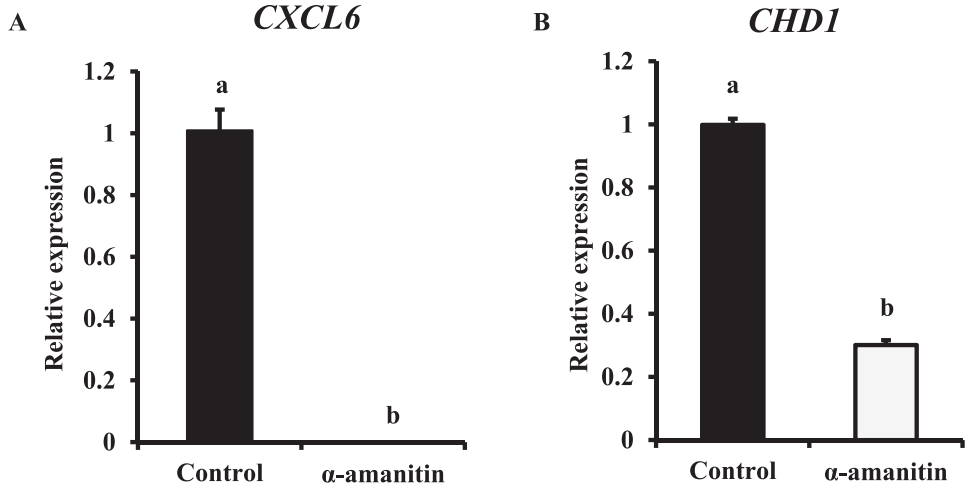

FIG. 2. *CHD1* mRNA is of both maternal and zygotic origin. Zygotes were cultured with or without α-amanitin (50 μg/ml), an RNA polymerase II inhibitor. After 52 h, embryos were sampled for qPCR (n = 4 pools of 10 embryos each per group) against the known zygotic transcripts, *CXCL6* (**A**) and *CHD1* (**B**). Data were normalized to endogenous control (*RPS18*) and are shown as mean ± SEM. Different superscripts indicate significant differences ($P < 0.05$).

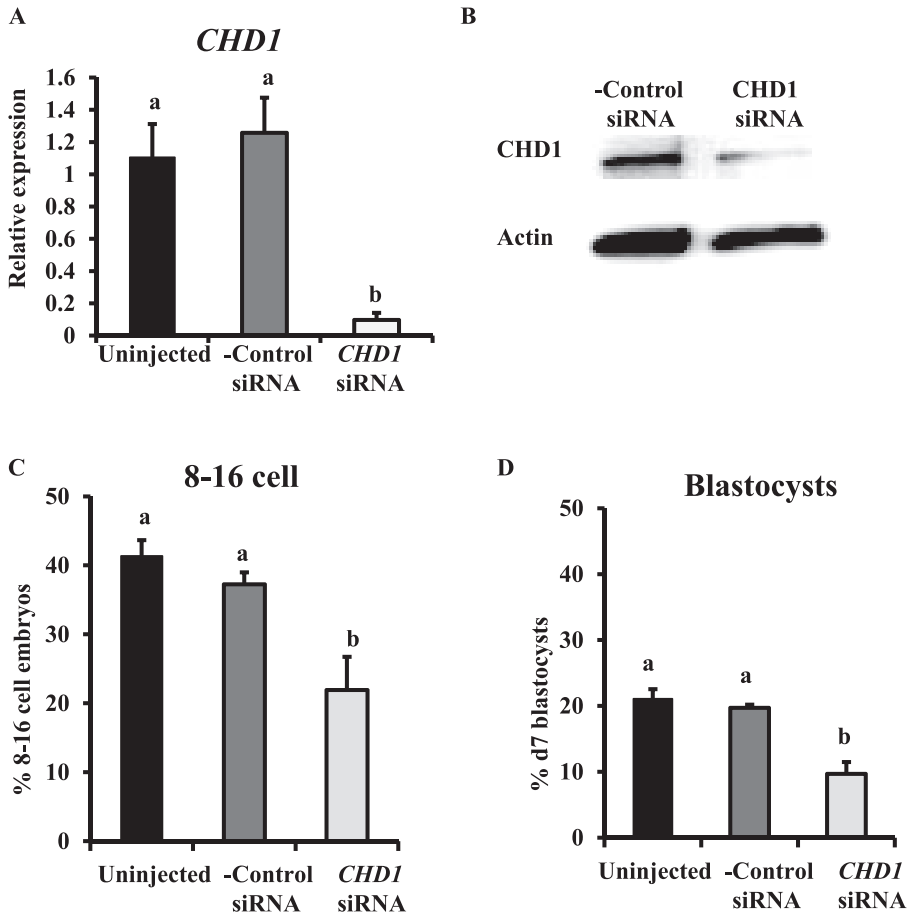

FIG. 3. CHD1 is required for bovine early embryonic development. **A** and **B**) Putative zygotes generated by IVF were injected (16–18 h postfertilization) with *CHD1* siRNA (25 μM, 20 pl) or negative control siRNA. Embryos were then sampled at 44 hpi (n = 3 pools of 10 embryos each per treatment) for qPCR analysis (**A**). Embryos were collected at 52 hpi and Western blot assay performed (n = 3 pools of 30 embryos each per treatment) (**B**). **C** and **D**) Presumptive zygotes were cultured in KSOM medium supplemented with 0.3% BSA (25–30 presumptive zygotes per group, 7 replicates). The 8- to 16-cell-stage embryos were separated at 72 hpi and cultured in fresh KSOM medium supplemented with 0.3% BSA and 10% FBS until Day 7. In vitro development for the following endpoints was monitored: (**C**) proportion of embryos developing to the 8- to 16-cell stage (72 hpi) and (**D**) proportion of embryos developing to the blastocyst stage (Day 7). Data in **A** were normalized to control (RPS18) and are shown as mean ± SEM. All percentages were calculated relative to the number of zygotes cultured for each treatment, and are expressed as mean ± SEM. Different superscripts indicate significant differences ($P < 0.05$).

shown). Nonetheless, the percentages of embryos developing to the 8- to 16-cell and blastocyst stages were both significantly reduced in response to *CHD1* ablations (Fig. 3, C and D).

CHD1 is required for maintenance of pluripotency in ESCs [9]. To examine the role of CHD1 in lineage differentiation in vivo, qPCR against lineage-specific markers was performed. Results showed that expression of *NANOG* (inner cell mass [ICM] marker) and *CDX2* (trophectoderm [TE] marker) was not changed (Fig. 4, A and B), suggesting that blastocyst cell determination was likely not affected by ablating CHD1.

CHD1 Ablation Reduces H3.3 Deposition in Bovine Early Embryos

Previously, maternal CHD1 was shown to be required for H3.3 incorporation onto male pronuclei in *Drosophila* [8]. Recently, several groups independently demonstrated the functional requirement of H3.3 in mouse preimplantation development and its involvement in various critical biological functions, including chromatin reprogramming [23–25]. Hence, we asked if H3.3 deposition is normal in *CHD1* knockdown (KD) embryos. Immunofluorescence analysis clearly showed that H3.3 was localized onto nuclei after fertilization, as expected. The intensity of H3.3 was reduced by 60% in *CHD1*-ablated embryos relative to controls (Fig. 5, A and B). To further determine the influence of *CHD1* ablation on chromatin configuration, immunofluorescence against well-documented posttranslational modification of histones was performed. However, no differences in H3K4me3 (Fig. 5, A and C) were detected between *CHD1* KD embryos and control groups.

H3.3 KD Results in Similar Phenotypes with CHD1 *KD in Bovine Embryos*

To determine if H3.3 defects account for the developmental failure of *CHD1* KD embryos, we designed siRNAs against H3.3 and tested effects of H3.3 knockdown in bovine early embryos. There are two genes encoding *H3.3*: *H3F3A* and *H3F3B*. Two effective siRNAs against each gene were generated. As shown in Figure 6, A and B, endogenous *H3F3A* and *H3F3B* were both greatly reduced by siRNA cocktail injection (two siRNAs/gene). Accordingly, H3.3 protein signal was decreased significantly, as detected by immunofluorescence analysis (Supplemental Fig. S4).

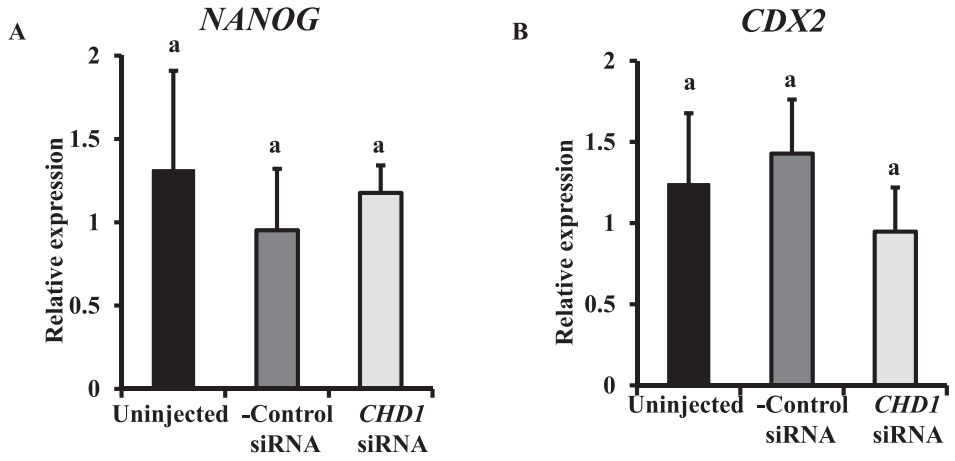

FIG. 4. Effect of *CHD1* knockdown on selected molecular markers of ICM and TE cell lineage determination in bovine blastocysts. Zygotes were microinjected with *CHD1* siRNA or negative control siRNA species 1, or served as uninjected controls. Blastocysts were collected on Day 7 (n = 4 pools of 3 embryos each per group) for qPCR analysis against the ICM marker, *NANOG* (**A**), and TE cell markers, *CDX2* (**B**). Data were normalized to endogenous control (*RPS18*), and are shown as mean ± SEM. Different superscripts indicate significant differences ($P < 0.05$).

It was shown previously that H3.3 ablation affects nuclear morphology in mouse preimplantation embryos [24]. In agreement with this report, the percentage of embryos containing micronuclei was greatly elevated after ablating H3.3 (Fig. 6D). Interestingly, our results also showed that *CHD1* KD results in increased proportions of embryos containing micronuclei (Fig. 6, C and D).

To further test if developmental potential of *H3.3* KD embryos mimics *CHD1* KD embryos, in vitro development of *H3.3* KD embryos was monitored. The percentage of embryos cleaved within 48 h was not affected (data not shown); however, the percentage of embryos developing to the 8- to 16-cell and blastocyst stages were reduced significantly at Days 3 and 7, respectively (Fig. 6, E and F).

DISCUSSION

Molecular regulation of chromatin remodeling during mammalian preimplantation development remains poorly understood, especially for agricultural animals. Identifying the relevant molecules and their regulation and regulatory role is critical, as early embryonic mortality is a major reason for infertility. In the present study, we have disclosed the functional role of CHD1 in bovine early embryonic development, and uncovered that CHD1 regulates early embryonic development, potentially by controlling deposition of critical histone variant, H3.3, into chromatin after fertilization. To our knowledge, our study is the first report presenting evidence supporting the involvement of CHD1 in H3.3 deposition in mammals.

Results show that both *CHD1* mRNA and protein abundance were greatly increased after meiotic maturation and were sustained at a relatively high level after fertilization, followed by a sharp decrease. Given that this increase occurs during a transcriptional quiescent period of development, it suggests that CHD1 may be an exception, with transcription beginning prior to the major wave of embryonic genome activation (EGA). This development period encompasses the time when the major wave of EGA occurs in cattle [26]. The *Chd1* mRNA profile described here in bovine is similar to the one in the mouse published recently [27]. More specifically, expression is high between the two- and eight-cell stages, and decreases significantly after that in the mouse. Considering that major wave of EGA occurs at the two-cell stage in mouse, this expression pattern suggests that *CHD1* in mouse embryos is also of maternal and zygotic origin. Consistent with this conclusion, our results show that *CHD1* expression was partially suppressed when embryos were exposed to α-amanitin, indicating both maternal and zygotic origin for *CHD1* in cattle (Fig. 3). However, in contrast with our Western blot results, protein level did not vary among different stages in the mouse, suggesting different posttranscriptional regulation between bovine and murine models. Interestingly, *CHD1* mRNA and protein were increased dramatically during oocyte maturation in cattle (Fig. 1, A and B). Given that transcription is generally quiescent during this period, further investigation of the biological role of *CHD1* in meiotic maturation is warranted.

In the present study, siRNA-mediated silencing of *CHD1* in bovine embryos led to a significant reduction in the proportion of embryos developing to the 8- to 16-cell and blastocyst stages compared with controls, indicating a functional contribution of CHD1 to bovine early embryonic development. Recently, genetic evidence showed that *Chd1*-null mouse embryos arrest at the peri-implantation stage (around E6.5) [10]. RNAi experiments in mouse preimplantation embryos revealed that CHD1 is required for further development after blastocyst formation [27]. These studies suggest that CHD1 is more important at a later stage during early development in the mouse compared to the bovine model. It is also possible that maternal CHD1 is sufficient to support preimplantation development in the mouse. Taken together, these results imply a species-specific role for CHD1 in preimplantation development.

The first cell fate decision is made during morula-blastocyst transition, when the inside cells develop into ICM cells and outside cells develop into TE cells. ICM cells differentiate into the embryo proper plus part of the placenta, while TE cells develop into placental tissue. Previous work demonstrated that CHD1 is required for maintenance of pluripotency in mouse ESCs [9], suggesting that CHD1 may be implicated in regulation of lineage differentiation. Indeed, microinjection of siRNA against *Chd1* into mouse zygotes resulted in suppression of *Oct4*, *Cdx2*, and *Nanog* expression in preimplantation embryos [27]. Nonetheless, we did not detect differences in expression of *NANOG* or *CDX2* in *CHD1*-ablated blastocysts (Fig. 4). This discrepancy may be attributed to differential mechanisms underlying the regulation of lineage-specific

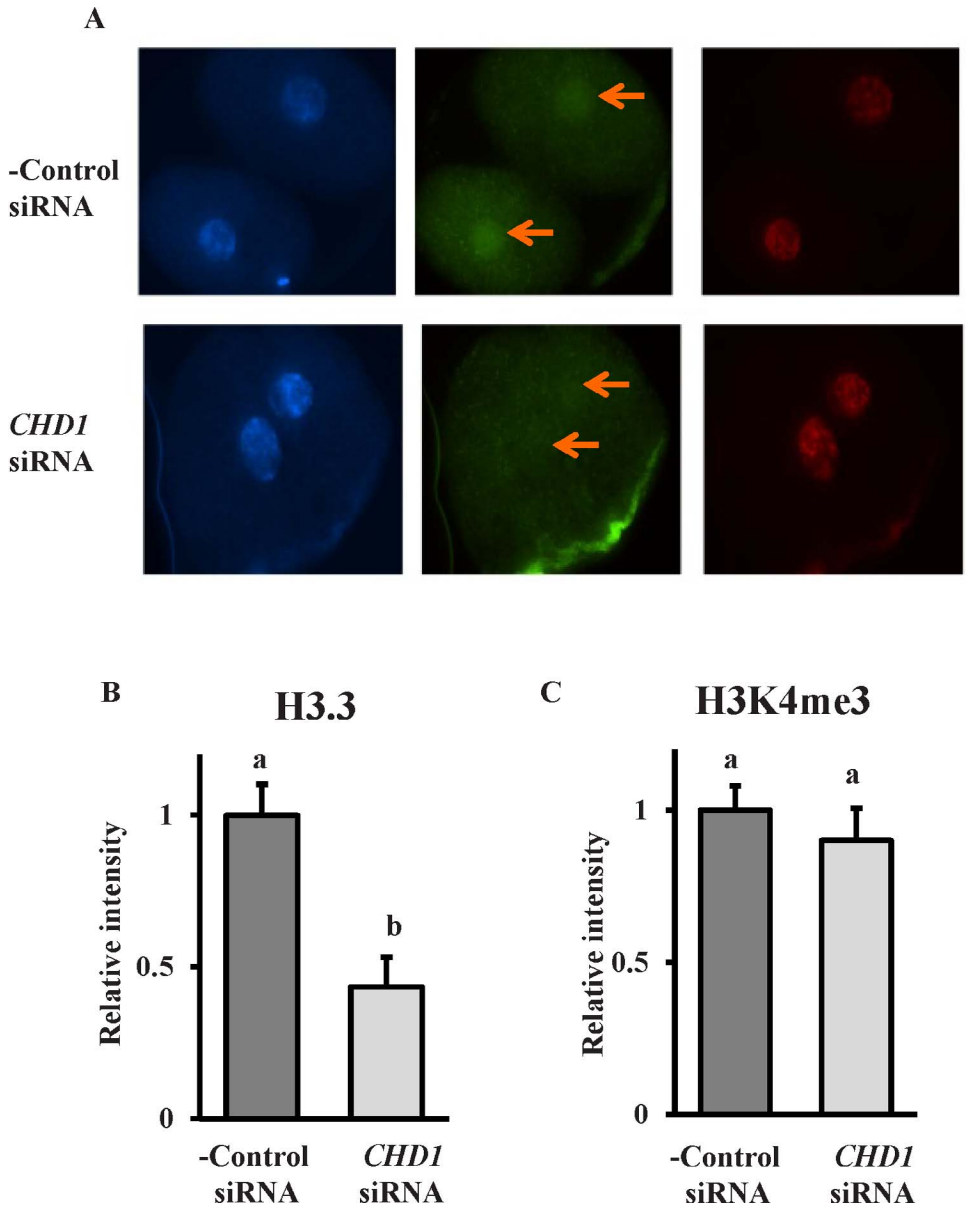

FIG. 5. H3.3 deposition is altered in embryos deficient in CHD1. Putative zygotes were microinjected with *CHD1* siRNA or negative control siRNA. Two-cell embryos were harvested for immunofluorescence analysis (**A**; n = 4 pools of 10 embryos/group). Arrows refer to nuclear area of blastomeres. Original magnification ×200. **B**) Quantitative analysis of fluorescence intensity of H3.3 and H3K4me3. Different superscripts indicate significant differences ($P < 0.05$).

factors between the bovine and murine models. In agreement with this conclusion, OCT4 and CDX2 are reciprocally inhibited in blastocysts of mice, but not cattle [28].

Previous work has shown that maternal CHD1 is necessary for H3.3 deposition into paternal pronuclei after fertilization in *Drosophila* [8]. However, it is still unclear if this association is conserved in mammals. Here, our results revealed that H3.3 signal intensity was dramatically decreased in *CHD1* KD embryos relative to controls. H3.3 is a crucial histone H3 variant, due to its preferential association with active chromatin [29]. Recently, several groups independently reported the functional requirement of H3.3 in mouse preimplantation development, which is mediated by regulation of heterochromatin formation, chromosome segregation, and epigenetic modifications immediately after fertilization [23–25]. Thus, we asked if H3.3 reduction mimics the developmental failure of *CHD1* KD embryos. Indeed, microinjection of *H3.3* siRNAs into bovine zygotes resulted in reduced development to the 8- to 16-cell and blastocyst cell stages. Additionally, *CHD1* KD led to increased proportions of embryos containing micronuclei, which was also found in *H3.3*-deleted embryos (Fig. 7C).

In conclusion, we are the first to demonstrate that CHD1 is involved in H3.3 deposition during preimplantation development in mammals. *CHD1* in bovine early embryos is not only transcribed from the maternal genome, but also from the zygotic genome. Ablation of *CHD1* from bovine early embryos causes a reduced developmental rate beyond the 8- to 16-cell and blastocyst stages. A greater understanding of the molecular control (e.g., CHD1, H3.3) of mammalian early embryo development will serve as the foundation on which future studies may be built to develop solutions to improve female fertility in both agricultural animals (e.g., dairy cows) and humans.

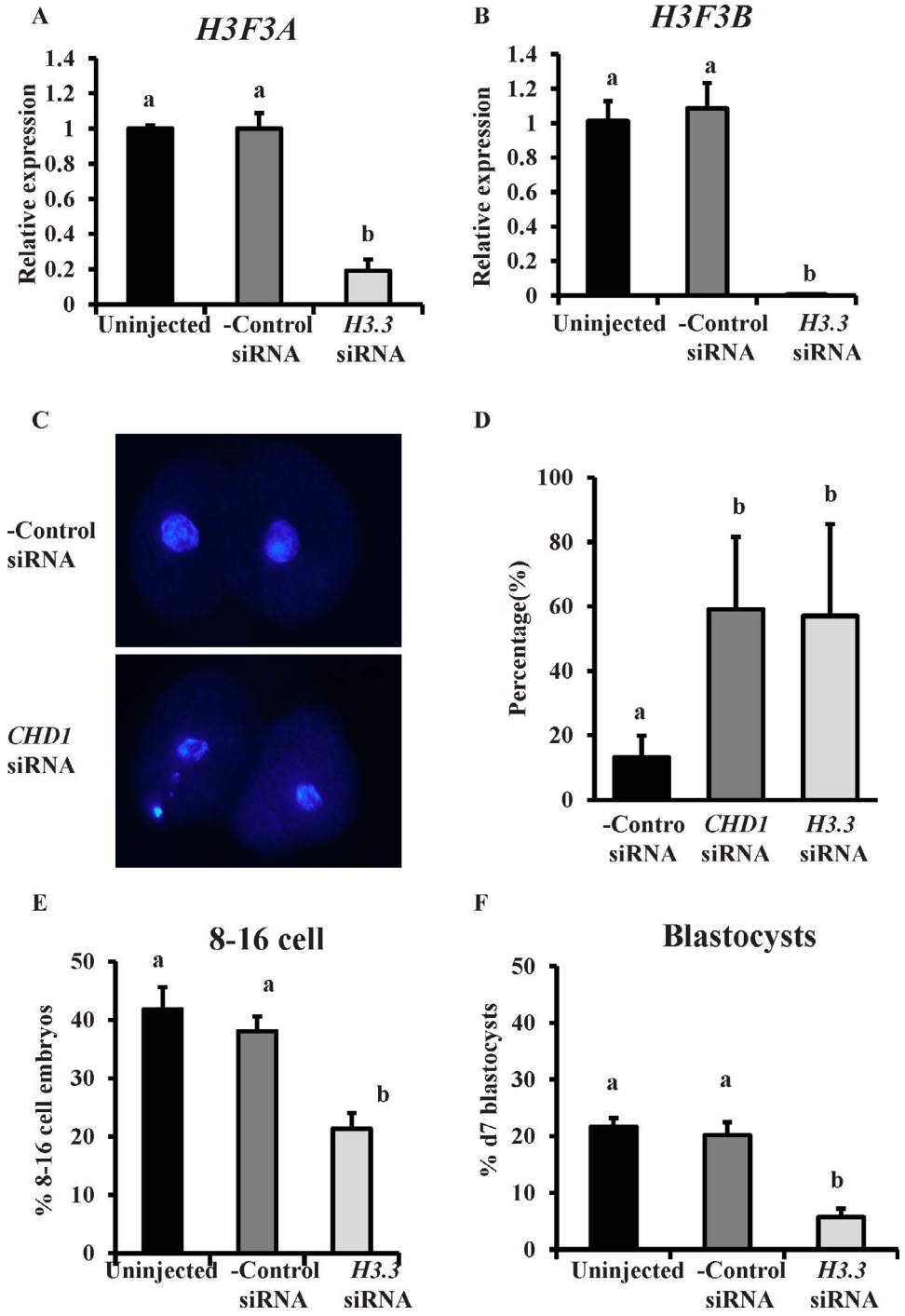

FIG. 6. *H3.3* knockdown phenocopies *CHD1* knockdown in bovine embryos. **A** and **B**) Validation of efficacy of *H3F3A* siRNA (**A**) or *H3F3B* siRNA (**B**) in targeting endogenous *H3F3A* or *H3F3B*. **C**) Increased frequency of micronuclei was found in *CHD1* knockdown embryos compared with controls. Original magnification ×200. **D**) Percentage of embryos containing micronuclei (n = 10 embryos; n = 3 replicates). **E** and **F**) percentage of embryos that develop to the 8- to 16-cell (**E**) and blastocyst (**F**) stages. Different superscripts indicate significant differences ($P < 0.05$).

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