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ARID1A, a component of SWI/SNF chromatin remodeling complexes, is required for porcine embryo development

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Abstract

Mammalian embryos undergo dramatic epigenetic remodeling that can have a profound impact on both gene transcription and overall embryo developmental competence. Members of the SWI/SNF (Switch/Sucrose non-fermentable) family of chromatin-remodeling complexes reposition nucleosomes and alter transcription factor accessibility. These large, multi-protein complexes possess an SNF2-type ATPase (either SMARCA4 or SMARCA2) as their core catalytic subunit, and are directed to specific loci by associated subunits. Little is known about the identity of specific SWI/SNF complexes that serve regulatory roles during cleavage development. ARID1A, one of the SWI/SNF complex subunits, can affect histone methylation in somatic cells; here, we determined the developmental requirements of ARID1A in porcine oocytes and embryos. We found *ARID1A* transcript levels were significantly reduced in 4-cell porcine embryos as compared to germinal-vesicle-stage oocytes, suggesting that ARID1A would be required for porcine cleavage-stage development. Indeed, injecting in vitro-matured and fertilized porcine oocytes with double-stranded interfering RNAs that target *ARID1A*, and evaluating their phenotype after seven days, revealed that the depletion of ARID1A results in significantly fewer cells than their respective control groups ($P < 0.001$).

Keywords

BAF; BRG1; epigenetics

1 Introduction

Extensive, dynamic epigenetic remodeling takes place during the first week of mammalian embryo development, which influences gene expression and developmental potential. Therefore, all epigenetic changes that impact chromatin structure and transcription must be coordinated to ensure proper embryo development.

A number of different epigenetic marks are remodeled during cleavage development. Trimethylation at lysine 27 of histone H3 (H3K27me₃), for example, is a covalent epigenetic mark that gradually decreases in abundance during early cleavage divisions in both bovine and porcine embryos (Ross et al, 2008; Park et al, 2009). Non-covalent changes

to chromatin structure are also utilized, whereby chromatin-remodeling complexes reposition and restructure nucleosomes in an ATP-dependent process (Bartholomew et al, 2014). Of the multiple ATP-dependent chromatin remodeling factors characterized, the SWI/SNF (Switch/Sucrose non-fermentable) complexes play a critical role during mammalian cleavage development (Klochendler-Yeivin et al, 2000; Wang et al, 2010).

SWI/SNF chromatin remodeling complexes are large and assembled from many components, including SMARCA2 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2; also known as Brahma [BRM]) or SMARCA4 (also known as Brahma-related gene 1 [BRG1]). SMARCA4 and SMARCA2 are ATPases that serve as the catalytic subunit of all SWI/SNF complexes. A series of BRG1-associated factors (BAFs) assemble with either SMARCA2 or SMARCA4 to create a functional SWI/SNF chromatin-remodeling complex. Which BAFs associate with a catalytic subunit determine the identity of a given SWI/SNF chromatin-remodeling complex, and dictate where that complex will act (Clapier and Cairns, 2009).

Extensive evidence suggests that SWI/SNF-mediated chromatin remodeling is critical for mammalian embryo development. Distinct SWI/SNF complexes do not appear to functionally overlap: In mice, *Smarca2*-null embryos develop to term and exhibit a mild overgrowth phenotype as adults (Reyes et al, 1998), whereas *Smarca4*-knockout embryos fail to progress further than the blastocyst stage (Bultman et al, 2000) and possess less H3K27me3, which could be linked to increased apoptosis and growth retardation (Singh et al, 2016). Indeed, SMARCA4 appears to regulate zygotic genome activation in mammalian embryos (Bultman et al, 2006), and serves a critical role in trophectoderm development in mouse embryos (Wang et al, 2010). SMARCA4 physically cooperates with CHD4 (Chromodomain helicase DNA binding protein 4), an epigenetic transcriptional repressor (Singh and Archer, 2014). Altered SMARCA4 expression can influence the methylation at lysine 4 of histone H3 (H3K4) (Glanzner et al, 2017), which negatively affects porcine embryo development and changes the abundance of other chromatin remodeling proteins (Magnani and Cabot, 2009).

BAF subunits possess unique functions within SWI/SNF complexes, and may be utilized in a cell-specific manner. SMARCC2 (BAF170), SMARCC1 (BAF155), and SMARCB1 (SNF5) appear to be involved in all SWI/SNF complexes, and support remodeling activity; their down-regulation results in cell cycle arrest and interrupted gene expression (Phelan et al, 1999). PHF10 (PHD finger 10) and ACTL6A (Actin-like 6A) appear to be required for neural stem and progenitor cells proliferation (Lessard et al, 2007). The expression of SMARCC1 and SMARCC2 also differs between undifferentiated and differentiated mouse embryonic stem cells, with SMARCC1 abundance being significantly higher than SMARCC2 in undifferentiated cells, but decreasing as stem cells differentiate (Yan et al, 2008). Finally, ARID1A (AT-rich interactive domain-containing protein 1A) and ARID2 are large, mutually exclusive BAF subunits (Lemon et al, 2001). The expression of *ARID1A*, *ARID1B*, and *SMARCA2* are up-regulated in rhesus monkey blastocyst-stage embryos, implying that these subunits function during cell lineage commitment (Zheng et al, 2004). ARID1A cooperates with Elongin C, Cullin 2, and Ring-box 1 to form an E3 ubiquitin ligase that functions as an adapter for remodeling of lysine 120 of histone H2B (H2BK120)

by the SWI/SNF complex (Xi et al, 2008). ARID1A also plays roles in the pluripotency of mouse embryonic stem cells as well as during early development, given that *Arid1a*-knockout mice fail to complete gastrulation (Wang et al, 2004; Gao et al, 2008; Li et al, 2010).

While SWI/SNF-mediated chromatin remodeling is clearly required for mammalian embryo development, which SWI/SNF complexes remodel chromatin during cleavage development remains unclear. Here, we tested the hypothesis that ARID1A is required for cleavage-stage development in the porcine embryo. We first quantified the changes in *ARID1A* transcript abundance in porcine oocytes and cleavage-stage embryos, and then performed an RNA interference (RNAi) assay to determine the developmental requirements of ARID1A during cleavage development. Our results revealed that *ARID1A* transcripts are in greatest abundance in germinal vesicle-stage oocytes, and decrease significantly in 4-cell-stage porcine embryos. We also find that knockdown of *ARID1A* results in impaired cleavage-stage development.

2 Results

2.1 Expression of ARID1A in porcine oocytes and cleavage-stage embryos

ARID1A transcripts were detected in porcine germinal vesicle-stage oocytes as well as 4-cell and blastocyst-stage embryos produced by in vitro fertilization or parthenogenesis. Porcine embryos produced by parthenogenetic activation are often used as a model for events that occur during cleavage stages, due in part to their ease of production and synchronous progression through early-cleavage stages. While parthenogenetic embryos are not always reliable proxies for studying developmental events that occur in biparental embryos, we wanted to test how similar the embryos produced by either fertilization or parthenogenesis might affect *ARID1A* abundance.

Germinal vesicle-stage oocytes possessed the highest abundance of *ARID1A* transcripts compared to 4-cell and blastocyst-stage embryos ($p < 0.05$). *ARID1A* transcript abundance was reduced 11.1- and 1.9-fold in parthenogenetic 4-cell and blastocyst-stage embryos, respectively, and 13.1- and 1.2-fold in embryos produced by fertilization at the 4-cell and blastocyst stage, respectively. No significant difference in *ARID1A* transcript abundance was found between 4-cell and blastocyst-stage embryos (Figure 1).

2.2 Efficiency of ARID1A knockdown

Germinal vesicle-stage porcine oocytes were assigned to one of three treatment groups immediately after cumulus cell removal: (i) *ARID1A* RNAi, which were injected with a short-interfering RNA (siRNA) that targeted *ARID1A*; (ii) control RNAi, which were injected with an siRNA containing a scrambled targeting sequence; and (iii) non-injected, which consisted of uninjected, denuded germinal vesicle-stage oocytes. Forty hours after treatment, RNA was isolated from each treatment group to determine the relative abundance of *ARID1A* transcript. A robust reduction in *ARID1A* transcript was associated with the *ARID1A* RNAi treatment (Figure 2).

2.3 Reduction in embryo development following ARID1A knockdown

Fertilized oocytes were assigned to one of three treatment groups five hours after gamete mixing: (i) *ARID1A* RNAi, wherein presumptive zygotes received an siRNA that targeted *ARID1A*; (ii) control RNAi, wherein presumptive zygotes received an siRNA containing a scrambled targeting sequence; and (iii) non-injected, consisting of uninjected presumptive zygotes. Immunocytochemical analysis of embryos revealed a marked reduction in ARID1A protein in embryos from the *ARID1A* RNAi group compared to the two control groups, which both possessed clear nuclear localization of ARID1A (Figure 3).

A significant reduction in the total cell number was observed in the *ARID1A* RNAi group compared to the two control groups (2.03 nuclei per embryo versus 4.95 and 5.58 nuclei per embryo for control RNAi and non-injected groups, respectively; $p < 0.001$) (Table 1). In addition, no embryos in the *ARID1A* RNAi group formed morphological blastocysts, – defined by the presence of a blastocoel in embryos 7 days after gamete mixing – whereas both control groups contained blastocysts (0% vs 5.8% and 6.7% for control RNAi and non-injected groups, respectively; $p < 0.05$). Indeed, only 2% of embryos in the *ARID1A* RNAi group developed beyond the 8-cell stage, whereas 17.9% and 19.4% of embryos in control siRNA-injected and non-injected embryos developed beyond the 8-cell stage (Table 1).

3 Discussion

Determining the expression and functional roles of chromatin remodeling subunits is central to understanding how epigenetic modifications are established and remodeled during critical developmental windows, such as zygotic genome activation and trophectoderm differentiation (Okada and Yamaguchi, 2017). Chromatin remodeling directed by the multi-subunit SWI/SNF remodeling complexes serves a critical role during these pivotal events of embryogenesis, (Bultman et al, 2006; Wang et al, 2010).

ARID1A is a unique subunit of the SWI/SNF chromatin remodeling complexes whose function is highly associated with cell cycle regulation and pluripotency (Kwon et al, 1994). For example, ARID1A participates in the repair of DNA double-strand breaks and sustains the function of ATR (Ataxia telangiectasia and Rad3-related protein), a member of the Phosphatidylinositol 3-kinase-like kinase family involved in DNA repair and cell cycle checkpoints (Shen et al, 2015; Watanabe et al, 2014). During cell proliferation and differentiation, ARID1A modulates SWI/SNF complex occupancy at specific loci involved with cell differentiation, such as *P19* in cardiac cells (Lei et al, 2015). In this study, we determined that *ARID1A* is highly expressed in germinal vesicle-stage porcine oocytes, but its transcript abundance declined in 4-cell-stage embryos; this expression profile is similar to the findings reported in mouse and rhesus monkey oocytes and embryos (Zheng et al, 2004; Gao et al, 2008).

Our observation that ARID1A is localized in the nuclei of 4-cell porcine embryos supports the hypothesis that ARID1A-containing SWI/SNF chromatin remodeling complexes play a crucial role during cleavage-stage development. Testing this function for ARID1A was achieved using an RNAi assay to deplete maternal *ARID1A* transcript in porcine zygotes, thereby creating a situation where *ARID1A* transcripts are reduced earlier. A well-

established in vitro fertilization protocol, utilized by our laboratory for over a decade, was employed in this study. Although the proportion of embryos that reached the blastocyst stage in our RNAi assay was low (~6%), the method itself historically results in a wide range of developmental potential, with 9%–28% of embryo reaching the blastocyst stage; indeed, in some publications, embryos generated by this method and transferred to recipients resulted in pregnancy (Wang et al, 2012; Park et al, 2012). Many factors contribute to the developmental capacity of in vitro-produced porcine embryos (e.g., source of the sperm, overall quality of the oocytes used in in vitro maturation, individual variability in handling technique). Despite the reduced overall development, the consistent experimental parameters used in this set of experiments to minimize variation still provided enough signal to observe a significant and robust change in developmental potential upon knockdown of ARID1A. Indeed, *ARID1A* knockdown starting in porcine zygotes decreased developmental competence. Thus, the pattern of ARID1A expression during early cleavage plus the outcome of RNAi assays collectively suggest that ARID1A may be necessary for complete zygotic genome activation, which occurs during the 4-cell stage in the porcine embryos (Kopecny 1989; Prather 1993).

In mice, ARID1A is required for complete development of the germ layers, given that *Arid1a* depletion in mouse embryos results in developmental arrest at the blastocyst stage (Gao et al, 2008). The difference in severity of the phenotypes of *Arid1a*-null mouse embryos versus porcine embryos subjected to RNAi-mediated *ARID1A* knockdown may be due to when targeted reduction was achieved. Null mouse embryos still possess maternal *Arid1a* transcript from the oocyte, but cannot produce de novo *Arid1a* transcripts; this maternal supply may be sufficient to support progress through early cleavage and zygotic genome activation, with lethality apparent as the embryo transitions to zygotic transcription of *Arid1a*. Conversely, the early depletion of maternal *ARID1A* transcripts in the porcine via RNAi results in an earlier phenotype, and revealed the possible contribution of this factor to early epigenetic remodeling of chromatin structure at targeted genes.

In conclusion, ARID1A is detectable in germinal vesicle-stage oocytes, and its abundance drops significantly at the 4-cell stage but increases again in blastocyst-stage embryos; this expression profile is shared across several species. Depletion or loss of ARID1A also negatively affects embryogenesis. Studies in humans and mice indicate that ARID1A is involved in the trimethylation of histones H2BK120, H3K4, H3K4, and H3K27 – all epigenetic marks that are critical in cell differentiation and cell proliferation, which are both essential for blastocyst formation (Zheng et al, 2004; Gao et al, 2008; Shen et al, 2015). Future experiments can be used to ask if the same is true in porcine embryos, employing chromatin immunoprecipitation experiments designed to measure changes in histone H3K9 or H3K27 methylation and/or H3K27 acetylation at discrete loci to provide insight into how repressive or active epigenetic marks change as ARID1A levels are altered during cleavage. Such data will help determine precisely how ARID1A directs the SWI/SNF complexes to specific loci and which genes are regulated in order to achieve proper embryo development.

4 Materials and Methods

4.1 Chemicals

All chemicals were obtained from Sigma Chemical Company (St. Louis, MO), unless stated otherwise.

4.2 Oocyte collection

Porcine (*Sus scrofa*) ovaries of prepubertal gilts were collected from a local abattoir, rinsed in 37°C saline, and transported to the laboratory in an insulated container. Cumulus-oocyte-complexes (COCs) and follicular fluid were manually aspirated from antral follicles (3-5 mm in diameter); these samples were held at 39°C and allowed to settle by gravity. COCs were resuspended in HEPES-buffered medium containing 0.01% polyvinyl alcohol (PVA) (Abeydeera et al, 1998). COCs with multiple layers of intact cumulus cells were selected for the experiments. COCs were vortexed in 0.1% hyaluronidase in HEPES-buffered medium for 9 minutes to remove the cumulus cells, resulting in denuded germinal vesicle-stage oocytes for PCR studies and microinjection.

4.3 In vitro maturation

COCs (90-120) were matured in 500 µl of tissue culture medium 199 (TCM-199) (Gibco BRL, Grand island, NY) containing 10 ng/ml Epidermal growth factor, 0.57 mM cysteine, 0.5 IU/ml Follicle-stimulating hormone, and 0.5 IU/ml Luteinizing hormone. COCs were matured for 42 hours at 39°C, 5% CO₂ in air, and 100% humidity (Aberdeera et al, 1998). Matured COCs were then vortexed in 0.1% hyaluronidase in HEPES-buffered medium containing 0.01% PVA for 4 minutes to remove cumulus cells.

4.4 Parthenogenesis

Denuded metaphase II-stage oocytes were placed between two platinum electrodes on an Electroculture chamber, and covered by activation medium (300 mM mannitol, 0.1 mM CaCl₂, 0.1 mM MgSO₄, 0.5 mM HEPES, 0.01% bovine serum albumin [BSA]). Oocytes were then artificially activated using two direct-current pulses of 1.2 kV/cm for 30 µsec provided by a BTX electro-cell Manipulator 200 (BTX, San Diego, CA).

4.5 In vitro fertilization and embryo culture

Thirty matured and denuded oocytes were placed in 100 µl of modified Tris-buffered medium (mTBM), and fertilized following an established protocol (Abeydeera et al, 1997), using fresh, extended boar semen. Semen was collected from a boar of proven fertility at the Purdue Animal Sciences Research and Education Center, extended in Modena Boar Semen Extender (Swine Genetics International, USA), and stored at 17.5°C for up to three days. Before fertilization, 1 ml of extended semen was mixed with 9 ml Dulbecco's phosphate-buffered saline containing 1 mg/ml BSA, and centrifuged for four minutes at 1000g at 25°C; this sperm washing was repeated a total of three times. The washed sperm pellet was resuspended in mTBM, and added to oocytes at a final concentration of 5×10^5 spermatozoa/ml; gametes were co-incubated for 5 hours at 39°C and 5% CO₂. Embryos were cultured at 39°C, 5% CO₂ in air, and 100% humidity in porcine zygote medium 3

(PZM3) supplemented with 3 mg/ml fatty acid-free BSA (Yoshioka et al, 2002) for 12 hours, 48 hours, and 7 days in order to collect pronuclear, 4-cell, and blastocyst-stage embryos, respectively.

4.6 RNA isolation, reverse transcription

RNA was isolated from porcine oocytes and embryos using the DYNABEADS reagent (Invitrogen Corporation, Carlsbad, CA, USA), according to the manufacturer's protocol. Briefly, pools of 100-150 germinal vesicle-stage oocytes, 4-cell embryos, and blastocyst-stage embryos were washed in HEPES-buffered medium three times, and placed in 100 μ l DYNABEADS lysis buffer; lysed cells were stored at -80°C (Lonergan et al, 2003). Reverse transcription was performed immediately after mRNA isolation using a 20- μ l reaction with the iScript kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer's protocol.

4.7 Quantitative PCR

4.7.1 Primers—Oligonucleotide primers were designed to amplify *ARID1A* using sequence data available in GenBank (XM_013988788.1). Oligonucleotide primers (forward, 5' - AGCCCAGAGATGATGGGTCT, and reverse, 5' - TCCTCCGTGAAGGCCAGATA) amplified a 209-base-pair product. The *ARID1A* PCR products were cloned into pENTRTM/SD/D-TOPO vector (Invitrogen, Carlsbad, CA, USA), and sequenced to confirm their identities. *ARID1A* primers were also used to construct standard curves. The amplification efficiency of the primer set was determined to validate our assay.

The abundance of *ARID1A* was determined relative to the housekeeping gene, *YWHAG* (GenBank accession number CO94522). Primer (forward, 5' - TCCATCACTGAGGAAACTGCTAA, and reverse, 5' - TTTTTCCTCACTCCGTGTTTCTCTA) amplified a 130-base-pair product (Whitworth et al. 2005).

4.7.2 Amplification—A master mix containing 12.5 μ l of SybrGreen Master mix (Bio-Rad), 5 μ l of each 1 μ M primer, and 2.5 μ l of each reverse-transcription reaction was prepared. For each replicate, cDNA from one pool of oocytes or embryos was separated into two groups: one to amplify *YWHAG* and one to amplify *ARID1A*; all reactions were performed on the same PCR plate. Reactions for each gene were run in duplicate, and three biological replicates were performed in total. The following amplification program was used on a MyiQ single-color real-time thermal cycler (Bio-Rad): 5 min initial denaturation at 94°C , followed by 45 cycles of 5 sec at 94°C , 30 sec at 60°C , and 30 sec at 72°C . A melt curve was produced to verify individual transcripts.

4.7.3 Quantification of transcript abundance—The comparative threshold cycle (Ct) method was employed to quantify the relative transcription abundance of *ARID1A* versus *YWHAG*, which was previously shown to maintain a constant level through preimplantation development in porcine embryos (Whitworth et al. 2005). The Ct value of *ARID1A* was subtracted from the Ct of *YWHAG* to obtain the change in Ct (ΔCt). The germinal vesicle-stage Ct was used as the calibrator to obtain ΔCt values. The *ARID1A* transcript

abundance at each embryonic was calculated using the 2^{-CT} method (Magnani and Cabot, 2008).

4.8 RNAi knockdown assays

4.8.1 Short-interfering RNA sequence—The BLOCK-iT™ RNAi Designer (Invitrogen) was employed to design the Stealth™ short-interfering RNA (siRNA) against *ARID1A* (5′ - CGGACAGCAUCAUGCAUCCUUCUAU), based on the full-length porcine *ARID1A* open reading frame. A nonsense Stealth™ RNAi siRNA (5′ - CGGCGACUAGUACUCCCUACUAU) was designed so it was not complimentary to *ARID1A*, yet maintained the same molecular weight as the *ARID1A* siRNA. Stealth™ RNAi nucleotides were diluted in RNase-free water to a concentration of 1 μM, and stored as 5-μl aliquots at −20°C.

4.8.2 Microinjection—Presumptive porcine zygotes were vortexed in 0.1% hyaluronidase in HEPES-buffered medium for 4 min to remove sperm immediately after gamete co-incubation. Presumptive zygotes were then divided into three groups in HEPES-buffered medium containing 3 mg/ml BSA: (i) *ARID1A* siRNA-injected, (ii) non-sense siRNA-injected, and (iii) non-injection controls. Injection needles were loaded with 5 μl of 1 μM siRNA, and then connected to an Eppendorf Femtojet microinjector (Eppendorf, Hauppauge, NY, USA). Embryos that lysed immediately after injection were removed and excluded from analysis. Surviving, injected embryos were placed in PZM3 embryo culture medium, and cultured for seven days. On the final day, embryos were subject to immunocytochemical staining, and nuclei were counted using a confocal microscope.

4.8.3 Efficiency of RNAi-mediated knockdown—Denuded germinal vesicle-stage oocytes were assigned to one of three treatment groups: (i) *ARID1A* siRNA-injected, (ii) non-sense siRNA-injected, and (iii) non-injection controls. All injected oocytes were placed in in vitro maturation medium for 40 hours, based on their respective treatment groups. mRNA was then isolated from intact oocytes from each treatment, and quantitative reverse-transcription PCR was performed.

4.9 Immunocytochemical staining

Porcine embryos cultured for seven days were collected and washed with HEPES-buffered medium three times, and fixed at 4°C for 1 hour in 3.7% paraformaldehyde. Following three washes in phosphate-buffered saline (PBS) containing 0.1% Tween-20 (PBST), fixed embryos were permeabilized for 1 hour using 1% Triton X-100 in PBS, and then incubated overnight (12-18 hours) in blocking solution (0.1 M glycine, 1% goat serum, 0.01% Triton X-100, 1% powdered nonfat dry milk, 0.5% BSA, and 0.02% sodium azide in PBS) (Prather and Rickords 1992). Embryos were then incubated overnight at 4°C with primary antibody against ARID1A (1:500 dilution of item ab182560 in PBST) (Abcam, Cambridge, MA, USA); washed three times in PBST (15 min per wash); and subsequently incubated overnight at 4°C with fluorescein isothiocyanate (FITC)-conjugated goat-anti-rabbit-IgG (diluted 1:500 in PBST). After three 15-min washes in PBST, embryos were stained with 5 μg/ml Hoechst 33342 for 20 minutes, and mounted on slides in Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA); slides were sealed with nail polish. Samples

were examined with an inverted Nikon A1R_MP confocal microscope, using de-scanned detectors and laser lines at 408 nm (Hoechst) and 488 nm (FITC).

4.10 Statistical analysis

The 2⁻ CT PCR values were imported into Statistical Analysis Software (SAS Institute, Cary, NC, USA), and analyzed with one-way analysis of variance (ANOVA). Differences were compared using Tukey's multiple comparison post-test. Embryo stages and replicates were considered as main factors, and $p < 0.05$ was considered significant. Two-way ANOVA model was employed in the microinjection experiments, and a multiple comparison between replicates and treatments test was performed in SAS.

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List of abbreviations in manuscript

ARID1A	AT-rich interactive domain-containing protein 1A
BAF	Brahma-related gene 1-associated factor
H2BK120	histone H2B lysine 120
H3K#[me3]	histone H3 lysine residue # [trimethylated]
RNAi	RNA interference
siRNA	short-interfering RNA
SMARC	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin
SWI/SNF	Switch/sucrose non-fermentable

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Figure 1.

ARID1A transcript abundance changes from germinal vesicle (GV)-stage oocyte to blastocyst-stage embryo. Fold expression was calculated by the 2^{-Ct} method, normalized to the abundance in germinal vesicle-stage oocytes. The average of three independent experimental replicates is shown. Different superscripts indicate significant differences between developmental stages ($p < 0.05$, by Tukey's multiple comparison post-test). For simplicity, the relative abundance of transcript in oocytes is shown twice (the same data were used for each bar at this stage) to more easily compare the relative abundance of *ARID1A* in embryos produced by parthenogenetic activation or in vitro fertilization.

Figure 2. Validation of RNAi-mediated *ARID1A* knockdown in porcine oocytes. Quantitative PCR results from germinal vesicle-stage oocytes 40 hours after injection of siRNAs targeting *ARID1A*. The fold abundance (2^{-Ct} method) is shown relative to the non-injected treatment. The average of two independent experimental replicates is shown.

Figure 3.

ARID1A protein levels are reduced in embryos injected with siRNAs that target *ARID1A*. Representative confocal images of nuclei from 4-cell-stage embryos from the three treatment groups: (i) *ARID1A* RNAi (a-b), (ii) non-sense control RNAi (c-d), and (iii) non-injected control (e-f). DNA and immunocytochemical staining for *ARID1A* are shown separately (*ARID1A* alone [b, d, and f]; DNA alone [a, c, and e]). Scale bar, 20 μm.

Table 1Knockdown of *ARID1A* in porcine embryos reduced in vitro developmental competence

Treatment	Morphological blastocyst	Average nuclear number	Total embryos examined
<i>ARID1A</i> siRNA	0	2.03 ^b	303
Control siRNA	19	4.95 ^a	329
Non-injected	17	5.58 ^a	252

^{ab} Superscript letters denote significant differences ($p < 0.0001$).