Original Article

CHD7 in oocytes is essential for female fertility

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Background: Chromodomain helicase DNA-binding protein 7 (CHD7), which is associated with CHARGE (Coloboma, Heart defect, Atresia choanae, Restricted growth, Genital hypoplasia and Ear abnormality) syndrome is an important regulator in many vital developmental processes. However, its role during oocyte development remains unknown.

Methods: We screened the Gene Expression Omnibus (GEO) database for expression levels of CHD7 during folliculogenesis. We generated a conditional knockout (cKO) mouse strain with oocyte-specific deletion of CHD7 (*Gdf9-Cre:Chd7*$^{f/f}$) using the Cre-loxP approach. Evaluation of follicle numbers and reproductive ability was then conducted. In addition, granulosa cell (GC) apoptosis was assessed by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay and cleaved caspase-3, using immunohistochemistry (IHC) and immunofluorescence (IF). GC proliferation was measured by Ki67 staining as evaluated by IHC.

Results: In our study, we demonstrated that CHD7 has high expression throughout all developmental stages of the oocyte. We found that deletion of *Chd7* in oocytes can cause infertility or sub-fertility in female mice and is associated with decreased follicle numbers at all stages. In addition, we found that GC apoptosis was significantly higher in cKO mice.

Conclusions: To our knowledge, our study has been the first to show that CHD7 plays a specific role during oogenesis. Our findings provide new insights into CHD7-related infertility.

Keywords: Chromodomain helicase DNA-binding protein 7 (CHD7); folliculogenesis; oocyte; fertility; reproduction

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Introduction

The chromodomain helicase DNA-binding protein 7 (CHD7) is an adenosine triphosphate (ATP)-dependent chromatin remodeler that plays pivotal functions during various developmental processes. ATP-dependent chromatin remodeling complexes consist of 4 subfamilies: the switching/sucrose nonfermenting (SWI-SNF) family, the imitation switch (ISWI) family, the CHD family, and the inositol-requiring 80 (INO80) family (1). These chromatin remodeling complexes share a similar ATPase-containing domain which can use ATP hydrolysis to alter histone-DNA contacts, exerting critical roles in normal development and cancer (2).

Previous studies have revealed that chromatin remodeling regulated by CHD7 is involved in key developmental processes at the transcriptional level, including neural crest cell formation, neuronal differentiation, great vessel, and cardiovascular development (3-6). CHD7 can regulate the transcription initiation and elongation at the enhancer and promoter region of the gene. For example, CHD7 can regulate neural differentiation in brain development by its transcriptional regulation in progenitor cells (7). Even heterozygous mutations of *CHD7* can cause a severe congenital disease known as CHARGE (Coloboma, Heart defect, Atresia choanae, Restricted growth, Genital hypoplasia and Ear abnormality) syndrome, with pathogenic mutations at the rate of approximately 60% to 70%. CHARGE syndrome is a rare disorder characterized by a specific pattern of defects, including ocular coloboma, heart malformations, atresia of the nasal choanae, growth retardation, genital hypoplasia, and ear abnormalities (8). Although CHD7-related CHARGE syndrome manifests in genital abnormalities, including cryptorchidism, micropenis, and abnormal morphology of female internal genitalia, its physiological roles in the female reproductive system remain less understood (9,10). Many researchers attribute these symptoms to a deficiency of gonadotropin-releasing hormone (GnRH)-induced gonadotropin secretion, which is caused by decreased GnRH neurons in the hypothalamus and decreased GnRH receptors in the pituitary gland (11). In patients with congenital hypogonadotropic hypogonadism (CHH), the percentage of CHD7 variants has been reported to be approximately 6–16% (12,13). CHH is also characterized by a deficiency in GnRH-induced gonadotropin secretions (14). These gonadotropin deficiencies can result in delayed puberty and agenesis of the uterus and ovaries (15).

However, the clinical and molecular genetic features of patients with *CHD7* allelic variants remain unclear. Firstly, not all patients with *CHD7* alterations and CHH symptoms respond well to cyclical gonadotropin therapy (16). In addition, patients with CHH have an almost 10% spontaneous recovery of their hypothalamic-pituitary-gonadal (HPG) axis function in later adulthood (17,18). So far, no recovery of the HPG axis has ever been reported in female patients with *CHD7* alterations and CHARGE, or in patients with *CHD7* alterations and CHH, indicating the complexity of the functions of CHD7 in the female reproductive system. Jolly *et al.* performed whole-exome sequencing analysis and reported that CHD7 was related to primary ovarian insufficiency (POI) (19). However, the role of CHD7 in ovarian function is not fully understood. To date, apart from studies on the effects on GnRH neurons, no reports have investigated the specific role of CHD7 in the reproductive system, especially during oogenesis.

Previous evidence from single-cell RNA-seq data has shown high expression levels of CHD7 during human oogenesis (20). In the present study, we examined a mouse strain of *Chd7-GFP* transgenic mice. In these mice, we observed high expression levels of *Chd7-GFP* in oocytes at different stages, from newborn to adult, which indicated that CHD7 might have a specific function in the ovary. To our knowledge, this is the first study to explore the function of CHD7 in follicle development. Due to the lethality of systemic *Chd7* knockout in mice, we could only conditionally delete *Chd7* in mice follicles using the Cre-loxP approach with the transgenic mice carrying growth differentiation factor 9 (GDF9) promoter-mediated Cre recombinase. This precise *Chd7* deletion in oocytes enabled us to explore the specific role of CHD7 during oogenesis, which could contribute to a better understanding of CHD7 function in the developmental stages of the female reproductive systems. We applied several important indicators to evaluate the ovary function like ovary size, fertility function, follicle counting. Small ovary size can give us the impression of the impaired ovary function and decreased ovarian reserve. Overall, we observed that conditional deletion of *Chd7* led to reduced ovary size, decreased reproductive ability, decreased follicle counting numbers. Furthermore, deletion of *Chd7* led to granulosa cell (GC) apoptosis during ovarian development, which is essential for normal oocyte formation. We presented the following article in accordance with the ARRIVE reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-609/rc).

Methods

mRNA expression profiling

The online website Gene Expression Omnibus (GEO) was used to explore the mRNA expression profiling of CHD7 (https://www.ncbi.nlm.nih.gov/geo/). In this study, we analyzed the CHD7 expression at different stages of human follicle development in GSE107746 and *Chd7* expression at different age of mouse germinal vesicle (GV) oocytes at 2, 9, and 14 months old from GSE159281. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Mice and genotyping

A protocol was prepared before the study without registration. All experiments were performed with the approval [No. 2019(135)] granted by the ethics committee of Children's Hospital of Fudan University, in compliance with the Guidelines for the Ethical Review of Animal Welfare and the Guide for the Care and Use of Laboratory Animals for the care and use of animals. All mice (n=40) had a C57BL/6J genetic background. *Chd7*$^{flox/flox}$ (*Chd7*$^{f/f}$) mice, *Chd7*-GFP transgenic mice, and *Gdf9*-Cre mice were obtained as previously described (21,22). Healthy female C57BL/6J mice were maintained as controls. All these mice were kept in pathogen-free conditions in an environment of 20±2 ℃ room temperature and 50–70% humidity, on a 12:12-hour light-dark cycle, with enough food and water. In the *Gdf9-Cre* mice, the Cre recombinase was specifically expressed in oocytes from primordial through to later stage follicles. As CHD7 is expressed in primordial follicle to mature oocytes, we used a *Gdf9-Cre* LoxP system to delete CHD7 in mouse oocytes. By crossing *Chd7*$^{f/f}$ mice with transgenic mice expressing GDF9 promoter-mediated Cre recombinase, we generated mice with no *Chd7* expression in their oocytes. Mouse genotyping was carried out using the universal mouse genotyping protocol adapted from Stratman and Simon (23). The following primers were used to detect the Cre allele: 5'-ACCAGGTTCGTTCACTCATGG-3' (forward) and 5'-AGGCTAAGTGC CTTCTCTACA'-3 (reverse). To detect CHD7 flox band, the following primers were used: 5'-TGCAGATGGGACGTTTTCAG-3' (forward) and 5'-CTGCAAGAACACAGGGCAAG-3' (reverse).

Fertility assessment and ovarian histology analysis

To assess fertility, 8-week-old *Gdf9-Cre:Chd7*$^{f/f}$ and *Chd7*$^{f/f}$ female mice were mated with normal 10-week-old wild-type (WT) males for 6 months (2 females: 1 male) (n=4 for each group). The number and size of the offspring from each pregnant female mouse were recorded at embryonic day 19 (E19) or after birth. For histological analysis, the ovaries were fixed in 4% paraformaldehyde for 24 hours at room temperature. After being embedded in paraffin, tissues were cut into 5–6 μm serial sections and stained with hematoxylin and eosin (H&E). The remaining sections were stored at room temperature for later use.

Follicle counting

For follicle counting, every follicle with an oocyte at a different developmental stage was counted in every fifth section of the ovaries. Only oocytes with a clear visible nucleus were counted, and follicle counts at 4 stages were recorded as means ± standard deviation (SD) per ovary, per section. Follicle classifications were defined as previously described (24). Primordial follicles were defined as those with oocytes surrounded by only a single layer of squamous GCs. Primary follicles were defined as those with oocytes surrounded by at least 3 cuboidal GCs in a single layer. Secondary follicles were defined as those with 2 or more layers of GCs around the oocyte but no antral space in the granulosa layer. Antral follicles were defined as those with at least 2 layers of GCs around the oocyte with an antral space. Follicle counting was performed blindly by two experienced analysts to reduce bias.

Real-time quantitative polymerase chain reaction (RT-qPCR)

TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Carlsbad, USA) was used to extract total RNA from ovaries in the different groups according to the standard protocol. cDNAs were synthesized using a PrimeScript RT-PCR kit (Takara Bio, Shiga, Japan). RT-qPCR was conducted on an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA) using a SYBR Premix Ex Taq II kit (Takara Bio). The primer sequences for *Chd7* were as follows: 5'-CAAAGCAGGGCCAGAACAAG-3' (forward), 5'-TCCCACGTGCTGTCTTCATA-3' (reverse). GAPDH

was used as an internal control for mRNAs with primers as follows: 5'-CCCATCACCATCTTCCAGGAG-3' (forward), 5'-CTTCTCCATGGTGGTGAAGACG-3' (reverse). Gene expression was analyzed using the ΔΔCq method.

Immunohistochemistry (IHC) assay

After slides were deparaffinized in xylene, tissue sections were rehydrated. This was followed by antigen retrieval. After washing in phosphate-buffered saline (PBS), tissue sections were labeled with antibodies against CHD7 (1:100, HPA053075, Sigma, St. Louis, USA), GFP (1:3,200, ab6556, Abcam, Cambridge, UK), cleaved Caspase-3 (1:200, 9661, Cell Signaling Technology, Danvers, MA, USA), and Ki67 (1:200, ab15580, Abcam). Slides were observed and photographed recorded under a microscope (400×) (Olympus, Tokyo, Japan).

Immunofluorescence (IF) assay

The removed ovaries were fixed in 4% paraformaldehyde and then immersed into 30% sucrose in PBS. After the ovaries were embedded in optimum cutting temperature (OCT) compound at –78 ℃, they were serially sectioned into 15 μm thicknesses in a cryostat (Leica, Wetzlar, Germany) for later use. After the slides were washed with PBS and blocked with 5% normal serum, the primary antibodies (GFP: 1:3,200, ab6556, Abcam; Ki67 1:200, ab15580, Abcam) were incubated at 4 degrees overnight. Expression was observed instantly using a Leica DMIRB Inverted Fluorescence Microscope (Leica, Wetzlar, Germany). The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) 1:5,000 (Solarbio, Beijing, China).

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

A TUNEL assay was performed on the ovary paraffin sections using an *in situ* cell death detection kit (11684795910, Roche, Basel, Switzerland) according to the manufacturer's instructions. The fluorescence signal was detected using a Leica DMIRB Inverted Fluorescence Microscope.

Statistical analysis

Data were presented as mean ± SD. Differences between the two groups were analyzed with *t*-tests. Multiple comparisons were analyzed with one-way analysis of variance (ANOVA) using GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA). A P value <0.05 was considered statistically significant.

Results

High expression levels of CHD7 in human and mouse oocytes

Firstly, using data from the GEO database, we investigated the expression level of *CHD7* during human folliculogenesis according to the gene expression profiles of GSE107746 (20). Single-cell RNA-seq revealed that CHD7 has a relatively high expression in oocytes from the primordial to the preovulatory stage, with especially high expression at the preovulatory follicle stage (*Figure 1A*). In contrast, the contemporary surrounding GCs had much lower *CHD7* expression levels. In another single-cell RNA-seq analysis evaluating the expression levels of *Chd7* in mouse oocytes and the surrounding cumulus at different ages, we obtained data showing high *Chd7* expression levels in mouse GV oocytes at 2, 9, and 14 months old from GSE159281 (*Figure 1B*) (25).

To evaluate *Chd7* expression during mouse folliculogenesis, we examined a *Chd7-GFP* transgenic mouse strain. Using IHC, we observed that *Chd7* had high expression from the primordial to preovulatory follicle stages in *Chd7-GFP* transgenic mice. (*Figure 1C-1E*). The expression pattern was similar in the IF results (*Figure 1F*). Overall, CHD7 had high expression levels in follicles at all stages but not in GCs. These results indicated that CHD7 might have a specific function in folliculogenesis.

Generation of Gdf9-Cre:Chd7$^{f/f}$ conditional knockout (cKO) mice

By crossing *Chd7$^{f/f}$* mice with transgenic mice expressing GDF9 promoter-mediated Cre recombinase, we generated *Gdf9-Cre:Chd7$^{f/f}$* cKO mice (*Figure 2A,2B*). In *Gdf9-Cre* mice, Cre was specifically expressed in oocytes in primordial and later-stage follicles from postnatal day 21 (26). In the present study, the PCR product sizes for genotyping were 610 bp (WT) and 750 bp (Chd7 flox allele) (*Figure 2C*). We used IHC to confirm the deletion of *Chd7* expression in the ovaries. Our results showed that CHD7 expression decreased in oocytes, confirming the successful generation of *Chd7* oocyte-specific cKO mice (*Figure 2D*). Total RNA was extracted from the whole ovary for RT-qPCR, which

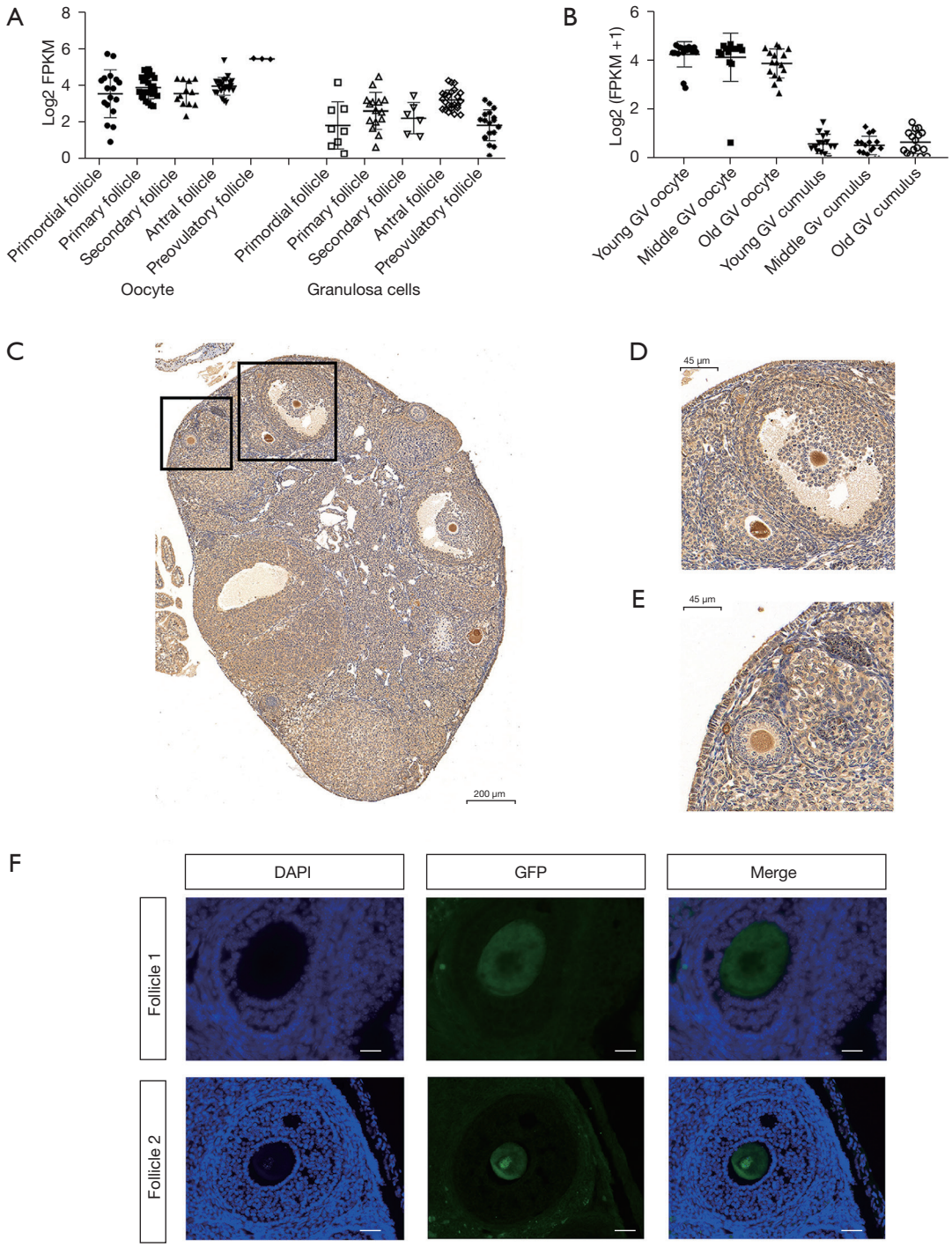

Figure 1 High expression levels of CHD7 in human and mouse oocytes. (A) CHD7 expression at different stages of human folliculogenesis. Data from the GEO database (GSE107746). (B) CHD7 expression in follicles at different mouse ages. Data from the GEO database (GSE159281). (C) Expression of GFP in *Chd7-GFP* transgenic mice, measured by IHC. (D,E) Higher magnification of the panels marked in (C). (F) Expression of GFP in *Chd7-GFP* transgenic mice, measured by IF (scale bar =50 μm). CHD7, chromodomain helicase DNA-binding protein 7; GEO, Gene Expression Omnibus; GFP, green fluorescent protein; IHC, immunohistochemistry; IF, immunofluorescence.

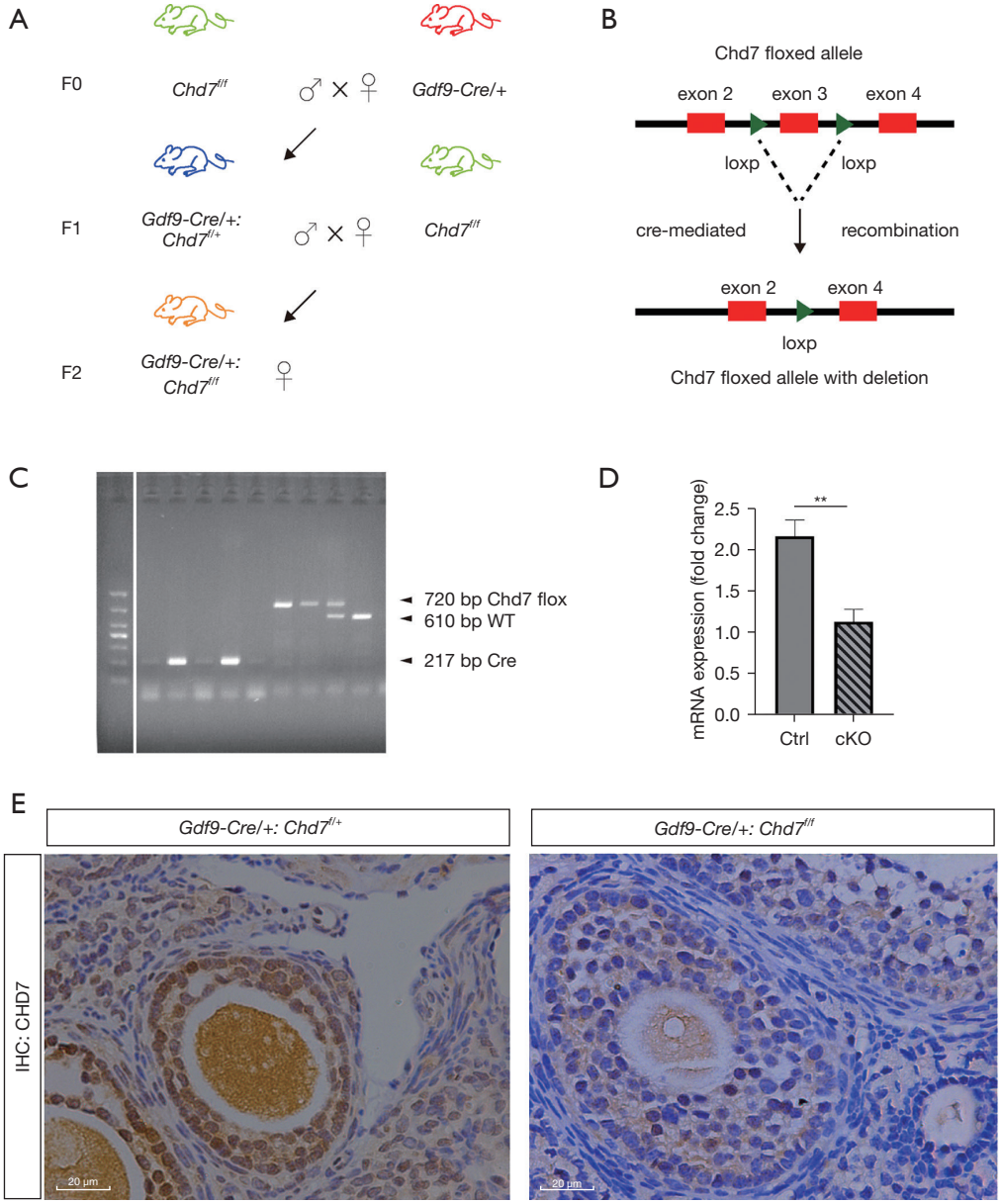

Figure 2 Generation of *Gdf9-Cre:Chd7*$^{f/f}$ cKO mice. (A) Mating scheme for genetically modified mice. (B) Schematic diagram of *Chd7* cKO mouse construction. (C) Genotyping for Cre and Chd7$^{f/f}$. (D) *Chd7* mRNA expression in cKO and WT mouse ovaries (E) CHD7 staining in cKO and WT mouse ovaries by IHC (scale bar =20 μm). **P<0.01. Gdf9, growth differentiation factor 9; Chd7$^{f/f}$, chromodomain helicase DNA-binding protein 7 flox/flox; cKO, conditional knockout; WT, wild-type; IHC, immunohistochemistry.

revealed a significant decrease in *Chd7* mRNA expression in the cKO mice compared to the control mice (*Figure 2E*).

Reduced fertility and decreased follicular numbers in cKO mice

We found that the cKO and WT mice had similar body sizes and weights at the age of 8 weeks. These cKO mice also showed normal behavior like WT mice without any abnormal phenotype. However, we observed that the cKO mice had significantly smaller ovaries and lower ovary weights compared to the WT mice (P<0.001) (*Figure 3A*). To test whether the deletion of *Chd7* had effects on female fertility, we performed a breeding test by

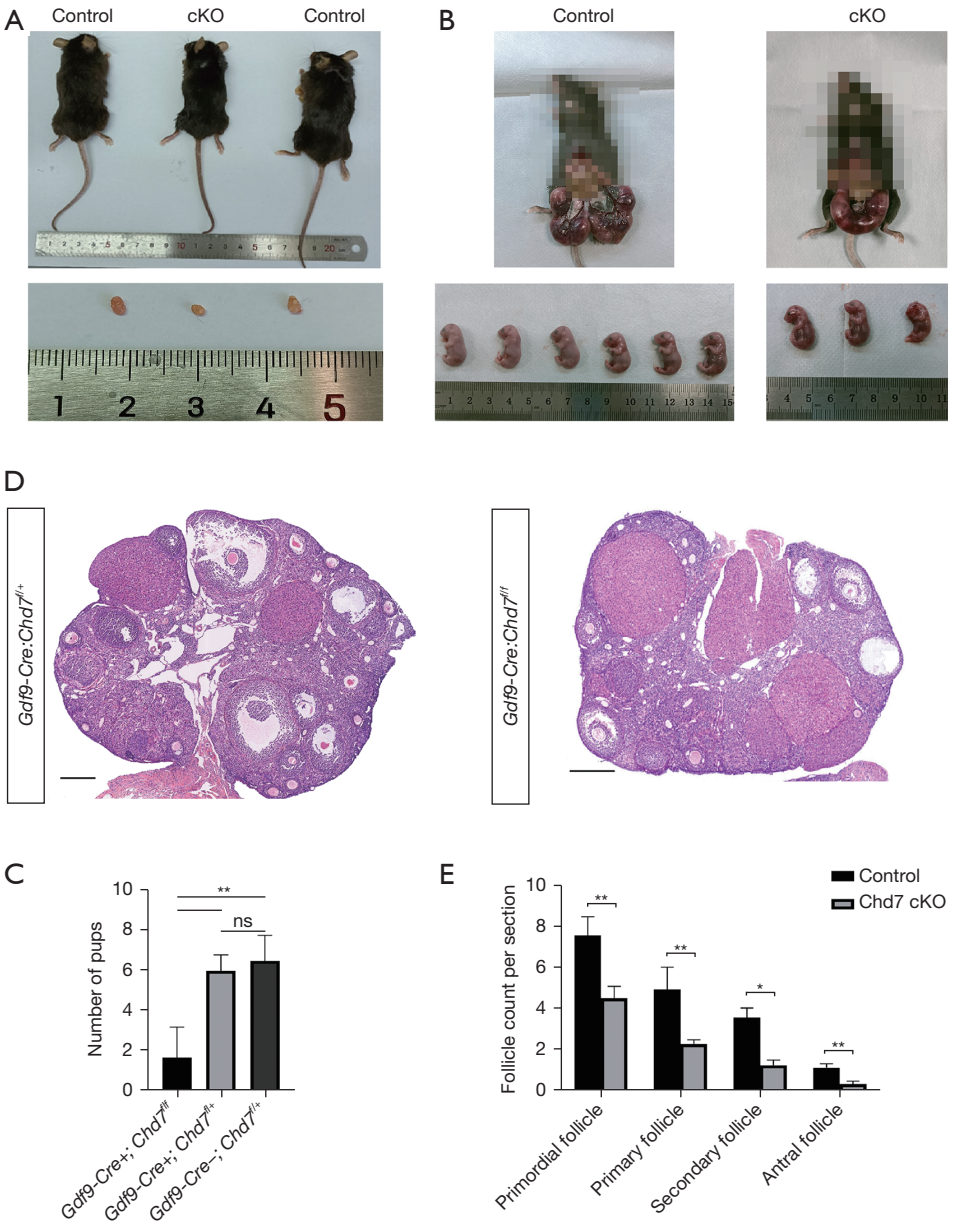

Figure 3 Reduced fertility and decreased follicular numbers in cKO mice. (A) Assessment of the bodies and ovaries of the cKO and WT mice. Significantly smaller ovaries were observed in the cKO mice, while no difference in body size was observed between the two groups. (B) Decreased fertility in the cKO mice compared to the WT mice. (C) The number of litters significantly decreased in *Gdf9-Cre+; Chd7*$^{f/f}$ mice compared to *Gdf9-Cre:Chd7*$^{f/+}$ and *Chd7*$^{f/+}$ (n=3 in each group). (D) H&E staining of the cKO and WT mouse ovaries (scale bar =250 μm). (E) Comparison of the numbers of follicles per section in different stages between control and cKO mice (n=3). Significantly decreased numbers of oocytes at all follicle stages were observed in the cKO mice. Data are expressed as mean ± SD. **P<0.01; *P<0.05; ns, not significant. cKO, conditional knockout; WT, wild-type; H&E, hematoxylin and eosin; Gdf9, growth differentiation factor 9; Chd7$^{f/f}$, chromodomain helicase DNA-binding protein 7 flox/flox.

mating female cKO and control mice with WT males for 6 months. We found that the number of litters was markedly reduced in the cKO mice compared to the control (WT or heterozygous genotype) females (*Figure 3B,3C*). The cKO female mice were completely infertile or sub-infertile, suggesting that CHD7 is essential for female fertility.

To further investigate possible defects in follicular development, we performed follicle counting in H&E-stained sections of the ovaries from control and cKO females. The H&E staining results allowed us to identify follicles at different stages in both genotypic groups (*Figure 3D*), and the follicle counting results revealed that the number of follicles at all 4 stages was markedly reduced in the cKO mice ($P<0.01$ in primordial, primary, and antral follicles, and $P<0.05$ in secondary follicles, n=3 for both groups) (*Figure 3E*).

CHD7 deficiency promoted GC apoptosis and did not inhibit cell proliferation

As ovary size was remarkably smaller in the cKO mice, we investigated whether CHD7 would affect apoptosis and proliferation levels in the ovaries. We performed a TUNEL assay and confirmed that the apoptosis rate was much higher in the GCs of the cKO mice than in the control mice, most notably in the preantral and early antral follicles (*Figure 4A*). The percentage of atretic follicles was significantly greater in the cKO mice than in the control mice. IHC indicated that the expression of cleaved caspase-3 positive cells in the cKO group was significantly higher than in the control group (*Figure 4B*). In addition, we used Ki-67 staining to detect cell proliferation in the cKO and WT mouse ovaries. IHC and IF results showed no difference between the cKO and control groups (*Figure 4C,4D*).

Discussion

ATP-dependent chromatin remodelers play critical roles in follicular development. These family members can change the chromatin structure by repositioning, mobilizing, and restructuring nucleosomes, using energy from ATP hydrolysis (27). Previous studies have confirmed that these family members have diverse functions during oogenesis. In 2006, Bultman *et al.* reported that depleting maternal BRG1 after oocyte development resulted in 2-cell arrest, thus revealing BRG1's regulatory function in zygotic genome activation (ZGA) in mice (28). Zhang *et al.* also reported that SNF2h (also known as Smarca5) plays a crucial role in oocyte maturation in mouse oocytes, showing that the loss of SNF2h could result in the dysregulation of several genes involved in maturation-promoting factor (MPF) activation (29). Another chromatin-remodeling factor, SNF21 (also known as Smarca1), has been shown to be required for oocyte superovulation and capable of regulating FGL2 in differentiating mouse GCs (30).

The CHD family includes the CHDs 1–9, which can regulate transcriptional activation and DNA repair (31). An important member of the CHD family, CHD7, is notorious for causing the recognizable genetic CHARGE syndrome. In addition, *CHD7* mutations are also associated with idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (32). CHD7-related reproductive phenotypes vary widely, from primary amenorrhea to delayed puberty and infertility. Previous studies have attributed these symptoms to a deficiency of hypothalamic GnRH (33,34). In recent years, as the understanding of the clinical features and molecular mechanisms of these *CHD7* alterations has improved, more researchers have realized the complexities of CHD7 function in the reproductive system. To date, most studies have not considered the specific function of CHD7 in folliculogenesis, independent of GnRH.

Our study attempted to determine the specific function of CHD7 in folliculogenesis. We first confirmed the high expression levels of CHD7 in mouse ovaries. Using *Chd7-GFP* transgenic mouse, we identified the expression patterns of CHD7 in the ovaries. With the creation of an approximately 200 kb-long bacterial artificial chromosome (BAC) containing the *Chd7* gene, we found that the *Chd7-GFP* mice analogized the expression pattern of the CHD7 gene in their ovaries. The BAC address for this line was RP23-127L15, which overlapped with the promoter and coding sequences of *Chd7*.

Transgenic mice, *Gdf9-Cre* and *Zp3-Cre*, are two common mice which allows inactivation of a target gene at different points during folliculogenesis. In the GDF9-Cre mice, the Cre recombinase was specifically expressed in oocytes from primordial through to later stage follicles (35). In Zp3-Cre mice, the Cre gene is driven by the zona pellucida protein 3 (Zp3) promoter which express the Cre recombinase in the oocytes at primary and/or later follicular stage (36). As CHD7 is expressed in primordial through to mature oocytes, we used a *Gdf9-Cre* LoxP system to delete CHD7 in mouse oocytes. After successfully generating the *Gdf9-Cre:Chd7$^{f/f}$* mice, we were able to further analyze the function of CHD7 during oogenesis.

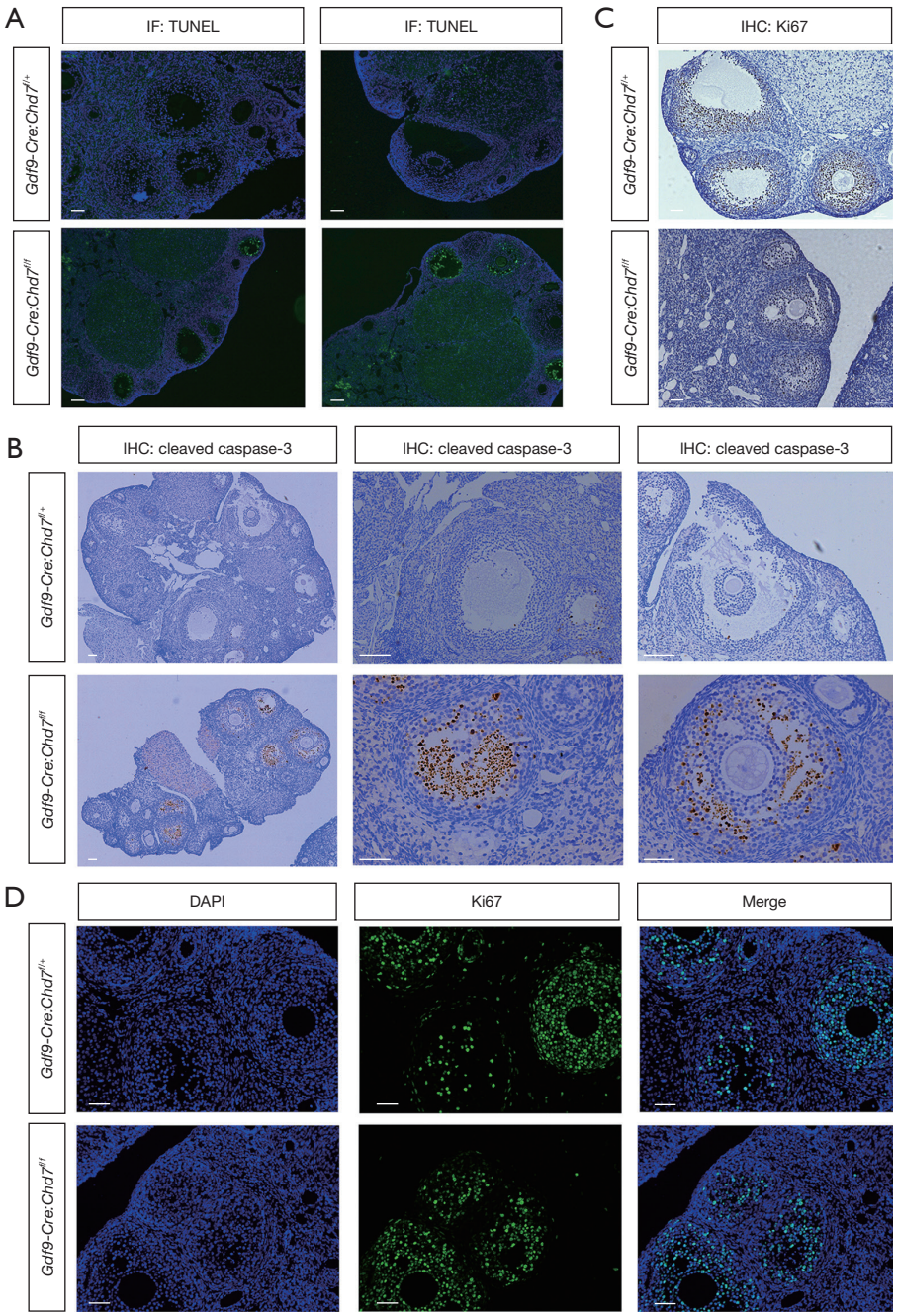

Figure 4 Higher GC apoptosis in cKO mice. (A) TUNEL assays showed higher GC apoptosis rates in an adult *Gdf9-Cre:Chd7*$^{f/f}$ ovaries compared with a *Gdf9-Cre:Chd7*$^{f/+}$ ovaries. (B) IHC staining was used to detect and compare cleaved caspase-3 expression in *Gdf9-Cre:Chd7*$^{f/f}$ ovaries and *Gdf9-Cre:Chd7*$^{f/+}$ ovaries. (C) IHC staining was used to compare Ki-67 expression between *Gdf9-Cre:Chd7*$^{f/f}$ ovaries and *Gdf9-Cre:Chd7*$^{f/+}$ ovaries. (D) IF staining was used to compare Ki-67 expression in *Gdf9-Cre:Chd7*$^{f/f}$ ovaries and *Gdf9-Cre:Chd7*$^{f/+}$ ovaries. (Scale bar =50 μm). TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; GC, granulosa cell; Gdf9, growth differentiation factor 9; Chd7$^{f/f}$, chromodomain helicase DNA-binding protein 7 flox/flox; IHC, immunohistochemistry; IF, immunofluorescence.

To our knowledge, our study was the first to observe Chd7-cKO mice showing infertility or sub-fertility, smaller ovary sizes, decreased preovulatory follicle numbers, and increased follicular atresia. These findings suggest that CHD7 could have a specific and important function in follicular development. In addition, we observed significantly higher GC apoptosis rates in the preovulatory follicles of cKO mice. The bidirectional interactions between oocytes and the GCs surrounding them are important for oocyte maturation because many steroidal hormones, like luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and growth factors, such as insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF) can benefit oocyte maturation and prevent cell apoptosis in preovulatory follicles (37,38). As a synchronous partnership, the relationship between the oocyte and its supporting GCs is vital (39). Oocytes can direct GC proliferation, differentiation, and apoptosis, while GCs can influence oocyte maturation.

Our preliminary results showed significant differences in apoptosis but not in proliferation of GCs between the cKO and control groups. The balance between pro-apoptotic and anti-apoptotic factors in GCs has been shown to be vital during folliculogenesis (40). Apoptosis of GCs can mediate follicular atresia, and our study found that greater follicular atresia in oocyte which is related with high apoptosis rates in *Chd7*-deficient mouse oocytes. Although the development of ovarian follicles is precisely co-regulated by pituitary-induced gonadotropins together with local paracrine factors in the ovary, the present study confirmed that CHD7 could exert a unique function in oocyte development by targeting the oocyte directly. Thus, we should consider that patients with *CHD7* alterations may not respond well to GnRH treatment.

This study had some limitations. First, we did not analyze the specific defects of these oocytes in the cKO mice. Secondly, as a chromatin remodeling factor, CHD7 regulates the transcription of many downstream genes through a process known as chromatin remodeling. More experiments are needed to explore the detailed mechanisms underlying this phenomenon.

Conclusions

To our knowledge, our study has been the first to confirm that CHD7 plays a crucial role in suppressing follicular atresia and promoting oocyte maturation.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-609/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-609/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-609/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All experiments were performed with the approval [No. 2019(135)] granted by the ethics committee of Children's Hospital of Fudan University, in compliance with the Guidelines for the Ethical Review of Animal Welfare and the Guide for the Care and Use of Laboratory Animals for the care and use of animals.

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