FGF2/FGFR signaling promotes cumulus–oocyte complex maturation in vitro

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Abstract

Fibroblast growth factor 2 (FGF2), a member of FGF family, binds with FGF receptors (FGFR) to initiate biological functions in various somatic cells. However, little is known regarding the role of FGF2/FGFR on oocyte meiosis. In this study, we investigated expression patterns and functions of FGF2/FGFR during in vitro maturation (IVM) of mouse cumulus-oocyte complexes (COCs). Among four FGFRs, Fgfr1 was the most abundant in COCs. The transcripts for Fgfr2 and Fgfr3 in COCs increased during IVM. Fgfr1 was present in oocytes and cumulus cells, while Fgfr2 was present in only cumulus cells. Treatment of COCs with the selective FGFR inhibitor SU5402 blocked oocyte meiotic progression and downregulated expression of Bmp15 and Gdf9. In contrast, supplement of FGF2 promoted oocyte meiotic progression and upregulated Bmp15 and Gdf9 expression. Inhibition of FGF with SU5402 reduced cumulus expansion and expressions of Ptx3, Has2 and Tnfαip6. Treatment with FGF2 increased Ptx3 and Has2 expression. Inhibition of FGFR had no effect on meiotic progression of denuded oocytes (DOs). However, co-culture of DOs with COCs or supplementation with FGF2 promoted meiotic progression of DOs. Inhibition of FGF2/FGFR signaling also downregulated Fgfr1 expression, while supplemental FGF2 upregulated Fgfr1 expression. Furthermore, inhibition of FGFR in COCs interrupted the c-Mos/MAPK pathway and maturation-promoting factor (MPF), as indicated by downregulation of oocyte c-mos and Ccnb1 transcripts, respectively. Overall, this study suggests that FGF2 produced by cumulus cells, activates a FGF2/FGFR autocrine/paracrine loop within COCs to regulate cumulus expansion and oocyte meiosis. These findings reveal a novel role for FGF2/FGFR signaling during in vitro maturation of COCs.

Introduction

Fibroblast growth factor 2 (FGF2), also known as a basic fibroblast growth factor (bFGF), is a canonical member of FGF family (Ornitz & Itoh 2015). FGF2 promotes follicular development in multiple species including the goat (Almeida et al. 2012), sheep (Santos et al. 2014), and macaque (Lu et al. 2015). Additionally, Mondal et al. (2015) reported that supplementation of maturation medium with FGF2 significantly increased the maturation rate of ovine COCs. Barros et al. (2019) suggested that the addition of FGF2 to IVM medium increased the percentage of oocytes reaching GVBD. The addition of FGF2 improved the efficiency of producing matured oocytes of pig (Yuan et al. 2017). Abouzaripour et al. (2018) indicated that the addition of FGF2 to the medium had beneficial effects on the IVM of mouse oocyte. Moreover, FGF2 could also improve early embryonic development (Zhang & Ealy 2012a, Abouzaripour et al. 2018). Collectively, these studies show that FGF2 can promote oocyte maturation; however, the cell types and underlying mechanisms of FGF2/FGFR regulation of oocyte meiosis remain unclear.

The biological functions of canonical FGFs are mediated by interactions with transmembrane receptors known as FGF receptors (FGFRs) (Zhang & Ealy 2012b). The FGFRs are tyrosine kinase receptors (TRKs) that are derived from four genes, namely Fgfr1, Fgfr2, Fgfr3, and Fgfr4 (Price 2016). FGF2 is expressed in caprine follicles (Almeida et al. 2012), in bovine cumulus cells (Barros et al. 2019), and in ovine oocytes and embryos (Santos et al. 2014, Mor et al. 2018). FGFR1, FGFR2, and FGFR3 are expressed in mouse oocytes and embryos (Lu et al. 2010). Previous studies demonstrate that FGF2 binds to and activates FGFR1 to carry out its diverse physiological functions in target tissues. For example, FGF2 promotes the proliferation of ovarian granulosa cells and germ cells through the activation of FGFR1 (He et al. 2012,
Collectively, these findings implicate FGF2 and FGFR in follicular development and oocyte maturation in mammals. However, the expression patterns of the ligand (FGF2) and receptors (FGFRs) in murine cumulus-oocyte complexes (COCs) have not been established. Furthermore, the role of paracrine trafficking of FGF2 between cumulus cells (CCs) and oocytes on oocyte meiosis has not been examined.

In the present study, mouse COCs, and isolated oocytes and cumulus cells, were employed to further explore the functions of FGF2/FGFR signaling on COCs during in vitro maturation (IVM). Specific objectives were to: 1) illuminate the expression patterns of FGF2 and FGFRs in COCs, 2) determine the effects of FGF2/FGFR signaling on oocyte meiosis and cumulus expansion, and 3) explore the possible mechanisms by which FGF2/FGFR signaling regulates oocyte in vitro maturation.

Materials and methods

Chemicals

The chemicals used in this study included FGF2 (Peprotech, USA), α-MEM (Gibco), M2 medium (Sigma), SU5402 (MCE, USA), penicillin-streptomycin (P/S) (Gibco), BSA (Genview, USA), PMSG (Sansheng, China), HCG (Sansheng, China), EGF (Peprotech, USA), sodium pyruvate (Sigma), and mineral oil (embryo-tested) (Sigma).

Animals

Female Kunming white mice (3 weeks old) were purchased from local Central Animal Laboratory (Wuhan, China). All mice were maintained under 12 h darkness:12 h light cycle with food and water supplied ad libitum at Huazhong Agricultural University animal facility. The animal handling procedures and all experimental protocols were approved by the Huazhong Agricultural University Animal Care and Use Committee (HZAUMO-2017-025).

Cumulus-oocyte complexes (COCs) collection

Female mice at 3 weeks of age were given an intraperitoneal injection of 10IU PMSG in sterile saline. After 46–48 h mice were euthanized by cervical dislocation. COCs were retrieved from ovaries by puncturing the follicles with needles in M2 medium. Only COCs containing compact cumulus cells were used for experiments. For experiments using denuded oocytes (DOs), the COCs were denuded of their cumulus cells mechanically by repeated pipetting using a fine-bore glass pipette in 0.1% hyaluronidase.

In vitro maturation (IVM)

The COCs were washed three times in maturation medium containing α-MEM supplemented with 1 IU/mL PMSG, 2 IU/mL HCG, 20 ng/mL EGF, 75 μg/mL penicillin, 50 μg/mL streptomycin, 0.2 mM sodium pyruvate and 3 mg/mL BSA. Then, 30–50 COCs were randomly allocated to each drop containing 80 μL of the appropriate culture medium covered with mineral oil and maintained at 37°C in 5% CO2 for 4 h or 14 h in vitro (Fig. 1A). The extent of meiosis in the oocytes was determined based on the ratio of germinal vesicle breakdown (GVBD) and the first polar body extrusion (PBE) (Liu et al. 2018, Rong et al. 2019). The maturation rate was calculated as the number of oocytes extruding the first polar body divided by the total number of oocytes cultured.

Treatment with FGF2 or the selective FGFR inhibitor SU5402

FGF2 was dissolved in ddH2O to prepare a 100 μg/mL stock solution and stored at −20°C before use. At the beginning of each culture, the stock solution was diluted with maturation medium to achieve a final concentration of 0, 1, 10, and 100 ng/mL FGF2. SU5402, a selective FGFR inhibitor, was dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C. At the beginning of each culture, the stock solution was diluted with maturation medium to achieve a final working concentration of 0, 5, 10, and 20 μM SU5402. The final DMSO concentration was less than 0.2%, which was determined to have no effect on the COCs in vitro maturation (Soto-Heras et al. 2019).

Assessment of the cumulus expansion and oocyte nuclear stage

After IVM, COCs were photographed with an inverted microscope, and the area of cumulus expansion was measured.
using Image J software. Subsequently, COCs were denuded of their cumulus cells using the method as previously mentioned, and then oocytes were classified as germinal vesicle (GV), germinal vesicle breakdown (GVBD) and metaphase II (MII). GVBD was determined visually by the disappearance of the oocyte nucleus, and the oocyte with visible the first polar body extrusion (PBE) was considered as a matured oocyte (metaphase II) (Fig. 1B).

Total RNA extraction and cDNA synthesis

Total RNA was extracted from mouse COCs (n = 120), DOs (n = 120), and cumulus cells (CCs, harvested from 120 COCs). Before or after IVM, COCs were collected, washed three times in PBS, and then transferred to a tube containing lysis buffer for RNA extraction. For experiments of extracting RNA from DOs and CCs separately, oocytes were denuded from cumulus cells using the method as mentioned previously, and then the resultant DOs and CCs were separately transferred into lysis buffer. All samples were stored at −80°C until RNA extraction. Total RNA was extracted using RNAprep Pure Micro Kit (Qiagen) in accordance with the manufacturer’s instructions. First-strand cDNA was generated using the cDNA synthesis kit (Thermo) following the manufacturer’s guidelines.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

To quantify RNA expression, sequence-specific primers were designed and synthesized by TSINGKE Biological Technology (Wuhan, China). Primer sequences were summarized in Table 1. Actb was selected as a reference gene (Acuna-Hernández et al. 2018). Real-time qPCR was performed using the CFX96 detection system (Bio-Rad) with SYBR Green (Qiagen) with the following program steps: 95°C for 2 min followed by 40 cycles of 95°C for 10 s, and 58°C for 30 s. The temperature was then gradually increased (0.5°C/s) to 95°C to generate the melting curve. For each group, qRT-PCR was performed in triplicate. Relative gene expression was calculated by the 2−ΔΔCt method.

Statistical analysis

Data sets were presented as the mean ± s.e.m. of at least three independent experiments. Data were analyzed using t-tests between two groups, and comparisons between three or more groups were made using one-way ANOVA followed by Tukey’s multiple comparison tests. P < 0.05 was considered statistically significant.

Results

The expression patterns of FGF2 and FGFRs during COCs in vitro maturation

The expression levels of the four FGFRs in COCs were determined using qRT-PCR. Fgfr1 expression level was the most abundant among the four FGFR receptors. The Fgfr1 expression level was 6500-, 150-, and 18,000-fold higher than Fgfr2, Fgfr3, and Fgfr4, respectively (Fig. 2A). To detect the expression profile of Fgfr1 in COCs during IVM, we examined the expression of Fgfr1 mRNA at three different time points, including 0, 4, and 14 h. The relative expression level of Fgfr1 mRNA in COCs increased approximately four-fold at 4 and 14 h, compared with 0 h (Fig. 2B). Fgf2 mRNA showed a similar pattern of expression as Fgfr1 in COCs during IVM (Fig. 2C). To determine which cell express Fgf2 and Fgfr1, cumulus cells and oocytes were harvested from pool of COCs, RNA was extracted, and Fgf2 and Fgfr1 expression was examined in each cell type. The results showed that Fgfr1 mRNA was expressed in both oocytes and cumulus cells, while Fgf2 mRNA was only present in cumulus cells (Fig. 2D).

FGF2/FGFR signaling regulates oocyte meiotic progression

To study whether FGF2 and FGFR interaction affect oocyte meiotic progression, we employed SU5402, a well-studied FGFR tyrosine kinase inhibitor (Fields et al. 2011, Zhang & Ealy 2012b). COCs were cultured in maturation medium supplemented with different concentrations of SU5402 (0, 5, 10, and 20 μM), and in vitro maturation of COCs were assessed by GVBD and the first polar body extrusion (PBE) rates. Supplementation

Table 1  Primer sequences of genes used for quantitative RT-PCR.

<table>
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<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
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<th>Fragment length (bp)</th>
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<td>GAGAAGACGCCACCCACAG</td>
<td>CACACTTAGGAAGCCAGCACG</td>
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<tr>
<td>Fgfr1</td>
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<td>GTCAGAAGGCACACAGCACTC</td>
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<td>Fgfr3</td>
<td>GGAGGACCGTGGCGAAGAC</td>
<td>CACAGGTTCAAGGAGGTCG</td>
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<td>103</td>
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<tr>
<td>Fgfr4</td>
<td>TCCATGAGCCTGCCTACAA</td>
<td>ATTTCAGATATTCGCAGGAC</td>
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</tr>
<tr>
<td>Bmp15</td>
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<td>153</td>
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<td>CTGAGGTAGTGGAGGCGAA</td>
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<td>Has2</td>
<td>GACGACAGGGCCCTTACCA</td>
<td>TGGCTTTGAGGGCGACGCA</td>
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of maturation medium with 20 μM SU5402 significantly decreased the GVBD rate compared with the control group (12.24 ± 14.44% vs 66.54 ± 9.40%, respectively) (Fig. 3A). In parallel, the maturation rate was significantly decreased in 20 μM SU5402 (5.26 ± 3.12%) compared with the control (78.59 ± 4.89%) and 10 μM SU5402 (61.07 ± 5.81%) (Fig. 3B). The reduction in GVBD and PBE induced by SU5402 was associated with significantly decreased expression of the oocyte maturation related genes Bmp15 and Gdf9 after 14 h of treatment (Fig. 3C).

These findings indicate that inhibition of FGFR suppresses oocyte meiotic progression in COCs. To further confirm the role of FGF2/FGFR signaling on oocyte meiosis, we tested whether exogenous FGF2 could promote oocyte meiotic progression. COCs were cultured for 4 h or 14 h with 0, 1, 10, and 100 ng/mL FGF2, and then GVBD and PBE rates were recorded. The result showed that addition of FGF2 to the maturation medium did not affect the percentage of oocytes reaching GVBD (Fig. 3D). However, significant improvement of maturation rate was observed in 100 ng/mL FGF2 treatment group (91.54 ± 7.71%) compared to the control (72.07 ± 0.49%) (Fig. 3E). FGF2 treatment (100 ng/mL) also significantly increased the expression of oocyte maturation related genes Gdf9 and Bmp15 at 14 h (Fig. 3F).

**FGF2/FGFR signaling regulates cumulus expansion**

To examine the effect of FGF2/FGFR signaling on cumulus expansion, mouse COCs were cultured in maturation medium supplemented with vehicle (Ctrl), SU5402 (20 μM) or FGF2 (100 ng/mL) and the area of cumulus expansion was assessed after 14 h. Cumulus cells (CCs) in the control group were completely expanded after culturing for 14 h (Fig. 4A, Ctrl). However, CCs remained compact in the SU5402 treatment group (Fig. 4A, SU5402). Quantification of the expansion area indicated that SU5402 treatment significantly prevented cumulus expansion (Fig. 4B). The morphological changes were further confirmed at mRNA levels through assessment of the relative expression of cumulus expansion related genes Ptx3, Has2, and Tnaiap6 (Matsumo et al. 2017). Consistently, the results showed that SU5402 treatment...
FGF2/FGFR promotes COCs IVM

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Our current data show that FGF2/FGFR signaling promotes IVM of COCs. Considering that FGFR1 was expressed in both cumulus cells and oocytes, we hypothesized that FGF2/FGFR signaling may also improve IVM of denuded oocytes (DOs). First, DOs were cultured in maturation medium supplemented with vehicle (Ctrl) or SU5402 (20 μM), then the rates of GVBD and the first polar body extrusion (PBE) were evaluated after 4 and 14 h, respectively. The rate of GVBD at 4 h and the rate of the first polar body extrusion (PBE) at 14 h were not statistically different between control and SU5402 treatment groups (Fig. 5A and B). Next, DOs were cultured in maturation medium with vehicle or 100 ng/mL FGF2. Exogenous FGF2 significantly improved the percentage of oocytes reaching GVBD at 4 h (66.70 ± 5.79% vs 75.60 ± 4.35%, Fig. 5C), but did not affect percentage of oocytes reaching MII at 14 h (54.68 ± 4.07% vs 55.85 ± 6.28%, Fig. 5D).

Considering that FGF2 is expressed by cumulus cells, a co-culture system employing denuded oocytes and COCs was developed (Emori et al. 2013) (Fig. 5E) to mimic the exogenous supplement of FGF2. We investigated whether co-culture DOs with COCs could improve the maturation of DOs. The result showed that the first polar body extrusion (PBE) rate of DOs in the co-culture group was significantly higher than that in the control group (55.25 ± 3.54% vs 79.52 ± 2.90%, Fig. 5F). Then, SU5402 was added into the denuded oocyte and COCs co-culture system to evaluate the maturation rate of DOs after 14 h. Treatment with SU5402 abrogated the ability of COCs to improve the maturation of DOs in the co-culture system (Fig. 5F).

To support the idea of the FGF2/FGFR autocrine/paracrine loop, we determined whether treatment COCs with exogenous FGF2 could stimulate the expression of the FGFR in COCs. FGF2 treatment provoked a nine-fold increase in Fgfr1 expression (Fig. 5G). Consistently, inhibition of the FGFR signaling by SU5402 in COCs significantly decreased (75% reduction) Fgfr1 mRNA expression (Fig. 5H). Furthermore, FGFR1 protein level was also significantly downregulated in COCs treated with the FGFR inhibitor (Fig. 5I). These data indicate that the FGF2/FGFR forms an autocrine/paracrine loop within COCs, which may facilitate oocyte maturation.

FGF2/FGFR signaling regulates MPF and MAPK activities

To explore the possible mechanism by which FGF2/FGFR signaling regulates oocyte meiotic progression, we determined the relative expression of transcripts for Ccnb1, a regulatory subunit of maturation/M-phase-promoting factor (MPF) and c-mos, a protein kinase that activates mitogen-activated protein kinase significantly decreased the expression levels of these three genes (Fig. 4C).

Addition of exogenous FGF2 had no visible effect on the morphology of COCs compared to the control group, which was understandable that cumulus cells were completely expanded in control group (Fig. 4D). Statistical analysis confirmed that addition of FGF2 only slightly promoted cumulus expansion area (Fig. 4E). However, FGF2 treatment significantly stimulated the expression levels of cumulus expansion related genes Ptx3 and Has2 (Fig. 4F).

Figure 4 Effect of FGF2/FGFR signaling on cumulus expansion. (A and D) The morphology of cumulus expansion in control group, 20 μM SU5402 treatment group, and 100 ng/mL FGF2 treatment group at 14 h. (B and E) Effect of 20 μM SU5402 (B) and 100 ng/mL FGF2 (E) on the area of cumulus expansion. The area of cumulus expansion was calculated using Image J software. Data are expressed as mean ± s.e.m. from at least three independent experiments. ***P < 0.001. (C and F) Effect of 20 μM SU5402 (C) and 100 ng/mL FGF2 (F) on the expression of cumulus expansion related genes Ptx3, Has2, and Tnaipl6 in cumulus cells at 14 h. Data are presented as means ± s.e.m. relative to Actb. *P < 0.05.
(MAPK), both of which contribute to oocyte meiotic progression (Kubiak 2013). Mouse COCs were cultured in maturation medium with vehicle or 20 µM SU5402. After 4 h of IVM, oocytes were denuded from cumulus cells, and the resultant oocytes were collected and used for detection of \( \text{Ccnb1} \) and \( \text{c-mos} \) expression. Treatment with SU5402 significantly decreased the expression of these two transcripts compared with the control group at 4 h (Fig. 6A and B), indicating that inhibition of FGFR regulates MPF and MAPK activities, required for oocyte meiosis.

**Discussion**

At least 23 fibroblast growth factors (FGFs) exist in mammals. FGF2 has received some recent attention because of its ability to regulate follicular development and oocyte maturation. Previous studies indicated that FGF2 could improve oocyte maturation rate in the sheep (Mondal et al. 2015), pig (Yuan et al. 2017), mouse (Abouzaripour et al. 2018), and blastocyst formation in the cow (Fields et al. 2011). Despite FGF2 function in IVM is widely reported, however, the FGFs function was achieved by binding to and activating a group of transmembrane-spanning tyrosine kinase receptors known as FGF receptors (FGFRs) (Fields et al. 2011, Zhang & Ealy 2012b). The FGF/FGFR signaling, and their relative expressions during IVM need to be addressed. Our present data showed that \( \text{Fgfr1} \) expression was the most abundant among the four FGF receptors (FGFR1-4) in mouse COCs. The transcripts for both \( \text{Fgf2} \) and \( \text{Fgfr1} \) increased during IVM of mouse COCs. Consistent with previous studies, the present study also showed that addition of FGF2 significantly facilitated the IVM of mouse COCs. In addition, exogenous FGF2 stimulated the expression of \( \text{Fgfr1} \) in COCs. The importance of FGF signaling was revealed by the use of a selective inhibitor FGFR, which significantly decreased the oocyte maturation rate and cumulus expansion. Inhibition of FGFR did...
not change the maturation rate of DOs, however, co-culture of DOs with COCs or exogenous FGF2 promoted the maturation of DOs, providing evidence that cumulus cells derived FGF2 can promote oocyte maturation. These results suggest that FGF2/FGFR signaling contributes to maturation of murine COCs in vitro.

Two approaches were taken to explore the role of FGF2/FGFR signaling on in vitro maturation of COCs. First, the maturation medium was supplemented with exogenous FGF2 to investigate the effects of FGF2 on meiosis in cumulus-enclosed oocytes. Our results show that FGF2 significantly accelerated oocyte meiotic progression, indicated by the improved rates of both GVBD and the first PBE. However, FGF2 did not increase total GVBD/PBE, which spontaneously reach a maximum with increasing time in culture (Rong et al. 2019). Besides, exogenous FGF2 induced transcripts for important oocyte-secreted factors, Gdf9 and Bmp15, which serve as reliable predictors of oocyte maturation (Tian et al. 2017, Belli & Shimasaki 2018). High levels of GDF9 in the follicular fluid are significantly correlated with oocyte maturation (Gode et al. 2011). To further test the function of FGF2/FGFR signaling on the oocyte meiosis, we employed SU5402, a selective inhibitor of FGFR. Although SU5402 has been described as a dual purpose inhibitor for vascular endothelial growth factor receptor (VEGFR) and FGFR, previous work showed that concentrations of SU5402 below 60 μM have no inhibitory activity on VEGFR, platelet-derived growth factor (PDGF), EGF or insulin signaling (Sugiura et al. 2007). Our results showed that SU5402 (20 μM) significantly suppressed oocyte meiotic progression and reduced Gdf9 and Bmp15 transcripts. These findings implicate FGF2/FGFR signaling in COCs IVM. Second, we evaluated the role of FGF2/FGFR signaling on cumulus expansion, which is considered as a critical indicator of oocyte maturation (Tian et al. 2017). In the present study, supplementation of the maturation medium with the FGFR inhibitor SU5402 significantly blocked cumulus expansion. Treatment with SU5402 also inhibited the expression of transcripts for cumulus expansion related genes, Has2, Tnaiap6 and Ptx3, indicating that FGFR receptor tyrosine kinase activity is required for cumulus expansion. Furthermore, treatment with exogenous FGF2 significantly increased the expression levels of cumulus expansion related genes Ptx3 and Has2. However, treatment with exogenous FGF2 had no visible change on cumulus expansion. It is well established that cumulus expansion proceeds spontaneously under IVM culture conditions. Because both cumulus cells and oocytes express FGFR1 and the cumulus cells express FGF2, it is reasonable to predict that exogenous FGF2 would have little additive effect on cumulus expansion. Taken together, our findings using two complimentary approaches (oocyte meiosis and cumulus expansion), we propose that FGF2/FGFR signaling plays a functional role in in vitro maturation of mouse COCs.

In the present study, we provide evidence for a FGF2 regulated autocrine or paracrine regulatory loop in mouse COCs maturation. Under standard IVM culture conditions, which do not include exogenous FGF2, inhibition of FGFR activity with SU5402 suppressed oocyte meiotic progression and cumulus expansion. Additionally, suppression of FGFR activity reduced the expression of Fgfr1 mRNA in COCs. Given that the IVM medium was fully defined (devoid of serum, containing 3 mg/mL BSA), it seems likely that endogenous FGF2 derived from cumulus cells contributes to COCs maturation. This idea is supported by observations that oocytes did not express Fgf2 mRNA and SU5402 did not impact oocyte meiotic progression of denuded oocytes (DOs). Previous studies showed that mouse oocytes could secrete FGF8, whose positive effect on oocyte maturation could be also inhibited by SU5402 (Sugiura et al. 2007, 2009). Therefore, the suppressive effect of SU5402 on IVM of mouse COCs in the present study may also involve inhibition of FGF8. Because FGFR1 is also expressed in oocytes, it is reasonable to suggest that exogenous FGF2 improved the rate of GVBD of denuded oocytes (DOs). Indeed, co-culture of DOs with intact COCs increases the rate of the first polar body extrusion (PBE), which was consistent with a previous report demonstrating that a DOs/COCs co-culture system is beneficial to the maturation of DOs (Emori et al. 2013). This evidence indicates that a factor from COCs contributes to DOs maturation. Furthermore, FGF2 directly stimulates GVBD in DOs, and SU5402 diminished the maturation of DOs in this co-culture system. Based on these data, we postulate that FGF2 produced by cumulus cells, activates FGFR in oocytes and cumulus cells, forming a FGF2/FGFR1 autocrine/paracrine loop within mouse COCs to regulate oocyte meiosis in vitro. However, it is worth noting that FGF2 derived from granulose cells could also contribute to the maturation of COCs in vivo (von Otte et al. 2006). Therefore, a FGF2/FGFR positive feedback loop may also promote follicle development in vivo. Studies in other FGF target tissues provide evidence for FGFs/FGFRs autocrine or paracrine regulatory loops (Buratini & Caixeta 2012, Hua et al. 2016). A previous study demonstrated that FGF10 derived from oocytes, binds to FGFRs in cumulus cells and oocytes to promote bovine oocyte maturation, cumulus expansion, and embryo development (Zhang et al. 2010).

Treatment of COCs with exogenous FGF2 increased the proportion of oocytes reaching metaphase II. However, FGF2 had no effect on the maturation rate of denuded oocytes (DOs). These indicate that the FGF2 effect on oocyte meiosis is likely mediated by cumulus cells and interactions with other FGFR/SU5402 sensitive signaling molecules produced via cumulus cells. It is also possible that other FGFs, such as FGF10 produced...
by cumulus cells, have positive effects on the maturation of oocytes (Zhang & Ealy 2012b). Besides, previous studies illuminated the critical role of the EGF signaling in integrating the interactions between cumulus cells and oocytes (Downs 1989, Kawashima et al. 2012, Sugimura et al. 2015, Yang et al. 2016). Cumulus cells express EGFR, which are activated by EGF that enables oocyte maturation in COCs (Shimada et al. 2016). Besides, FGF and EGF signaling cooperate with oocyte-derived members such as GDF9 and BMP15, which are involved in the maturation of oocyte (Sugiura et al. 2009). Additionally, Fgfr2 expression is significantly increased in cumulus cells after eCG treatment (Furukawa et al. 2014), which could promote the actions of endogenous FGF2 as reported in the present study. Therefore, it is likely that FGF signaling crosstalk with EGF and/or eCG signaling cooperate to facilitate oocyte maturation.

Oocyte maturation requires various signaling pathways. MPF (M-phase Promoting Factor) and MAPK (Mitogen-activated protein kinase) are two major kinases driving oocyte maturation (Kubiak 2013). The c-mos proto-oncogene product, a serine/threonine kinase, is a strong activator of MAPK pathway, and the cyclin B1 protein is an essential regulatory subunit of MPF. It is reported that oocyte meiosis progression requires cyclin B1 synthesis (Gao et al. 2014, Pereira et al. 2019). In the present study, we showed that FGFR inhibition with SU5402 significantly decreased the transcripts for Ccnb1 and c-mos. These findings support the idea that disruption of FGFR affects oocyte meiotic progression by interfering with key meiotic maturation signals.

In conclusion, the present study reveals that FGF2/FGFR forms an autocrine or paracrine loop within COCs to promote mouse COCs in vitro maturation, that is, cumulus expansion and oocyte meiosis. FGF2 produced by cumulus cells binds with FGFR expressed in cumulus cells to stimulate the expansion of cumulus cells. Additionally, FGF2, as well as other FGF ligands (such as FGF8, FGF10, etc.) can bind with FGFR expressed in the oocyte to promote oocyte meiosis by affecting MPF and c-Mos/MAPK activities (Fig. 7).

Declarations of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
C Du participated in the experimental design, performance of experiments, data analysis, figure preparation and the manuscript draft. J-S Davis, L-G Yang participated in the experimental design and manuscript revision. C Chen, Z Li, Y Cao, H Sun performed experiments. B-S Shao, Y-X Lin, Y-S Wang helped to analyze data. G-H Hua conceived, designed and coordinated the study and revised the manuscript. All authors read and approved the final version of the manuscript.

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