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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Fibroblast growth factor 2 regulates cumulus differentiation under the control of the oocyte

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Abstract

Purpose We first assessed regulation of *FGF2* expression in cumulus cells by FSH and oocyte-secreted factors during in vitro maturation (IVM). Then, we tested the hypothesis that FGF2 regulates meiotic progression, cumulus expansion, and apoptosis in cumulus-oocyte complexes (COC) undergoing IVM.

Methods In vitro maturation of bovine COC was utilized as a model to assess regulation of *FGF2* expression by FSH and oocyte-secreted factors (via microsurgical removal of the oocyte), as well as effects of graded doses of FGF2 on meiotic progression, degree of cumulus expansion, dissociation of cumulus cells, and cumulus cells apoptosis. Expression of genes regulating functional endpoints altered by FGF2 treatment was assessed in cumulus cells by real-time PCR. Cultures were replicated 4–5 times and effects of treatments were tested by ANOVA.

Results *FGF2* mRNA expression was increased by FSH and oocyte-secreted factors during IVM. Addition of FGF2 to the IVM medium advanced meiosis resumption, decreased the ease with which cumulus cells were dissociated, and inhibited cumulus cells apoptosis. Decreased cumulus dissociation was accompanied by decreased expression of *TNFAIP6*.

Conclusions This is the first study showing that *FGF2* expression is regulated by the oocyte in cumulus cells. Moreover, we report novel effects of FGF2 on cumulus cell survival and extracellular matrix (ECM) quality during IVM that may favor acquisition of developmental competence and suggest physiological roles during the final steps of COC differentiation.

Keywords FGF2 · IVM · Oocyte-secreted factors · Meiosis · Cumulus expansion · Apoptosis

Introduction

Due to its limited efficiency, oocyte in vitro maturation (IVM) has been basically restricted to patients with polycystic ovary syndrome (PCOS). Nevertheless, the optimization of IVM concentrates great interest in order to reduce the risk of ovarian hyperstimulation syndrome associated with current hormonal protocols that stimulate follicle development and induce oocyte in vivo maturation, as well as to reduce treatment cost making assisted reproduction more accessible [1]. Current IVM systems do not faithfully mimic the environment where the cumulus-oocyte complex (COC) differentiates in vivo, which results in suboptimal or impaired cumulus cells metabolism, asynchronous oocyte nuclear and cytoplasmic maturation, and decreased developmental competence [1]. Therefore, uncovering the physiological players and mechanisms underlying oocyte maturation is critical for improving the efficiency of IVM. In the face of the difficulty to obtain human samples for basic studies focusing the final differentiation of the COC, the importance of mono-ovulatory animal models as the bovine has been recognized [1].

In vitro, resumption of meiosis and cumulus expansion are usually stimulated by FSH, which stimulates the production of epidermal growth factor (EGF)-like peptides in cumulus cells in mice and cattle [2, 3]. The EGF-like peptides, including betacellulin (BTC), amphiregulin (AREG), and epiregulin (EREG), release meiotic maturation and promote cumulus expansion via ERK1 and 2 phosphorylation [4, 5]. Meiotic resumption is caused by the inhibitory action of ERK signaling on gap junction-mediated flow of cGMP from cumulus

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cells to the oocyte [5]. Granulosa and cumulus derived natriuretic peptide precursor C (NPPC) is also a key regulator of meiosis as it drives cGMP production by activating the natriuretic peptide receptor 2 (NPR2) in cumulus cells [6, 7]. In parallel, EGF-induced ERK signaling increases the expression of prostaglandin-endoperoxide synthase 2 (*PGTS2*), together with other genes involved in the production and organization of the extracellular matrix (ECM). These include hyaluronan synthase 2 (*HAS2*), that catalyzes the synthesis of hyaluronan (HA), tumor necrosis factor-induced protein 6 (*TNFAIP6*), and pentraxin (*PTX3*; [4, 8–11]).

In cattle, hyaluronan (HA) is the major component of the cumulus matrix, which stability and viscoelastic properties rely on cross-linking of HA with heavy chains (HC) of the inter-α-inhibitor protein (IαI) originated from the liver [12]. Tumor necrosis factor-stimulated gene 6 protein (TSG-6), encoded by the *TNFAIP6* gene, catalyzes the transfer of IαI HC onto HA forming HC-HA covalent complexes, which can then further crosslink with PTX3 to stabilize ECM structure [8, 13–15]. Also important for ECM organization are versican (VCAN), another cross-linking protein, and the HA surface receptors CD44 and RHAMM [16, 17]. Apart from serving as a cross-linking protein and as an anchorage point for HA, VCAN and CD44 may also transactivate EGF receptors thus enhancing ERK signaling [18]. The cumulus matrix formed during IVM appears to differ from that which forms in vivo, which stability may contribute to the generally lower developmental competence of IVM oocytes [19, 20]. It is of interest to identify factors that may improve ECM function and benefit fertilization in vitro.

Apart from EGF-like factors, another potential intrafollicular factor that is stimulated by the preovulatory LH surge in the cow is fibroblast growth factor 2 (FGF2) [21]. Although FGF2 is predominantly expressed by theca cells [22], mRNA and protein levels increase transiently in follicles/cumulus cells during the preovulatory period and during IVM in cattle [21, 23, 24]. Moreover, expression of FGF2 receptors in bovine cumulus cells is drastically increased by FSH during IVM, suggesting that sensitivity to FGF2 is enhanced in preparation for ovulation [2]. Within the follicle, FGF2 is best known to increase proliferation and inhibit apoptosis in granulosa cells [25–28], but it has also been shown to favor blastocyst formation in cattle and pigs in vitro [29–31] and therefore may act to enhance COC function, including cell health, cumulus expansion, and meiotic resumption.

Considering that FGF2 signaling appears to be upregulated by the LH surge in cumulus cells in cattle [2, 21], and that addition of FGF2 to the IVM medium benefits in vitro embryo production [29–31], we aimed to further investigate the regulation of *FGF2* expression in bovine cumulus cells, as well as potential roles of FGF2 during final COC differentiation possibly accounting for greater developmental competence. The first objective was to assess the regulation of *FGF2* mRNA levels by FSH and oocyte-secreted factors in cumulus cells during IVM, and the second objective was to determine the effects of FGF2 on oocyte meiotic progression, cumulus expansion and apoptosis in COC undergoing IVM.

Materials and methods

Unless where specified, all chemicals and reagents were purchased from Sigma (St. Louis, MO, USA).

Ovaries and COC culture

Ovaries of adult cows (predominantly Nellore, *Bos indicus*) were obtained from a local abattoir and transported to the laboratory within 2 h in sterile saline solution (0.9% NaCl) maintained at 26 °C. COC were aspirated from 3 to 8 mm diameter follicles with an 18-gauge needle and pooled in a 15 mL conical tube. After sedimentation, COC were recovered and selected using a stereomicroscope. Only COC with homogenous cytoplasm and multiple compact layers of cumulus cells were used. COC were washed and transferred in groups of 20 to a drop of basic maturation medium: TCM199, containing Earle's salts supplemented with 22 μg/ml sodium pyruvate, 75 μg/ml amicacin, 4 mg/ml BSA, and porcine FSH (Folltropin-V Bioniche Animal Health, Belleville, ON, Canada) at concentrations specified below for each experiment. Drops were incubated at 38.5 °C in 5% CO2 in humidified air.

Regulation of *FGF2* mRNA during IVM: Effects of time, FSH and oocyte-secreted factors

To assess the effect of time in culture on *FGF2* mRNA abundance, 20 COC were cultured in drops of 100 μL of maturation medium covered by mineral oil and containing 10 ng/mL of FSH (equivalent to 2×10^{-5} UI/mL) for 0, 4, 8, 12, 16, and 20 h (4 replicates). Then, to assess the effect of FSH concentration on *FGF2* mRNA abundance, 20 COC were cultured for 12 h in 100 μL drops of maturation medium supplemented with graded concentrations of FSH (0, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} μg/mL (equivalent to 0, 2×10^{-7}, 2×10^{-6}, 2×10^{-5}, 2×10^{-4} UI/mL; 4 replicates). After culture, cumulus cells were recovered to assess *FGF2* mRNA abundance by real-time RT-PCR.

Oocytectomy (microsurgical removal of the oocyte from the COC) was performed to test the effects of the oocyte on *FGF2* mRNA expression in cumulus cells from COC subjected to IVM induced with either FSH. Groups of 20 intact COC, oocytectomized COC (OOX) and oocytectomized COC in the presence of denuded oocytes (OD; 1 oocyte/μL) were cultured in 96 wells plates in 100 μL of base medium containing FSH (0.002 UI/mL) for 4 h (4 replicates), to assess if the oocyte can acutely regulate *FGF2* expression, and for 22 h (5 replicates), to assess longer-term regulation of FGF2 expression. For

oocytectomy, COC were placed in 200 μl drops of TCM199 partially covered with mineral oil and the cytoplasm of the oocytes was removed with a micromanipulator as previously described by Lima et al. [32]. After culture, cumulus cells were recovered to assess *FGF2* mRNA abundance by real-time RT-PCR.

Effects of FGF2 on oocyte nuclear maturation, cumulus expansion, and cell survival

To assess the effects of FGF2 on parameters of oocyte nuclear maturation, cumulus expression, and cell survival, groups of 20 COC were cultured in 450 μL of IVM basic medium supplemented with 1 μg/mL of FSH (equivalent to 2×10^{-3} UI/mL) as routinely done in our laboratory [32, 33], and graded concentrations of FGF2 (0, 1, 10, and 100 ng/mL).

To assess the effects of FGF2 on germinal vesicle breakdown (GVBD) and maturation dynamics, COC were subjected to IVM for 6 (5 replicates) and 22 h (8 replicates). After culture, oocytes were denuded mechanically by repeated pipetting in PBS, fixed in methanol 60% overnight at 4 °C, stained for 15 min with 5 μl Hoechst 33342 (Roche, Indianapolis, USA) and analyzed under UV light using epifluorescence microscopy. Oocytes cultured for 6 h were classified as GV (germinal vesicle) or GVBD and oocytes cultured for 22 h were classified as metaphase I (MI) or telophase I/anaphase I/metaphase II (TI/MII).

To examine the effects of FGF2 on degree of cumulus expansion, dissociation of cumulus cells reflecting matrix quality, cell survival, and apoptosis markers, IVM was performed as described above for 22 h in 5 replicates. The degree of cumulus expansion was visually classified according to a subjective scoring system: grade 1—poor expansion, characterized by a few morphological changes; grade 2—partial expansion, characterized by fair expansion but notable clusters lacking expansion; grade 3—complete or nearly complete expansion [34]. After evaluation of cumulus expansion, the ease with which cumulus cells dissociate was assessed by counting the number of pipetting movements necessary to completely denude the expanded COC after IVM, which was performed by the same individual throughout the study. Cumulus-oocyte complexes were denuded in groups of 20 and one pipetting movement was comprised of pipetting up and down actions using the volume of 80 μL.

To assess the effects of FGF2 on cell survival and apoptosis, cumulus cells were mechanically separated by repeated pipetting in drops of 100 μL PBS at the end of IVM, and then transferred to a 5-mL tube containing 100 μL PBS with 50 μg/mL propidium iodide (PI - BD Pharmingen, Mountain View, CA, USA; 0,5 μg/mL), 100 μg/mL Hoechst 33342, 0.2 μL CellEvent™ caspase 3/7 reagent (Thermo Scientific, Wilmington, DE, USA), and 2 μL of Annexin V-APC (BD Pharmingen, Mountain View, CA, USA). The resulting solution was incubated at 37.5 °C for 15 min, and then analyzed in a flow cytometer (BD LSR Fortessa, Mountain View, CA, USA). The data were analyzed by the software FACSDiva™ V6.2.

Assessment of mRNA abundance

To assess the effects of FGF2 on abundance of mRNA encoding proteins that control oocyte nuclear maturation, cumulus expansion, and apoptosis, graded doses of FGF2 were supplemented during IVM for 6 h (5 replicates). This time point was chosen in order to detect mRNA changes compatible with the observation of functional effects of FGF2 observed at 22 h of IVM. The experimental design to examine the effects of time, FSH, and oocytectomy on *FGF2* mRNA levels in cumulus cells is described above. For all experiments measuring mRNA abundance, at the end of culture cumulus cells were mechanically separated from COC by repeated pipetting in PBS. Cumulus cells were then transferred to 1.5 mL tubes, collected by centrifugation for 5 min at 700 G, and total RNA was extracted using the RNeasy® kit (Qiagen, Mississauga, ON, Canada) as recommended by the manufacturer. After purification, RNA samples were eluted in 30 μL of RNAse-free water and total RNA concentration measured by spectrophotometry using a NanoDrop ND® 1000 (Thermo Scientific, Wilmington, DE, USA). Total RNA (100 ng/reaction) was incubated with DNAse I (1 U/mg; Invitrogen, Sao Paulo, Brazil) and then reverse transcribed using Oligo-dT primers and Omniscript reverse transcriptase (Qiagen, Mississauga, ON, Canada).

Relative real-time RT-PCR analysis was performed with an ABI 7500 thermocycler using Power Sybr Green PCR Master Mix (Applied Biosystems, Sao Paulo, Brazil). The final volume of the PCR mix was 25 μL and thermocycling conditions were 95 °C for 10 min (1 cycle), denaturing at 95 °C for 10 s followed by annealing for 1 min (40 cycles). Sequences of primers, amplicon sizes, and annealing temperatures for all target genes are given in Table 1. Primers were as previously validated [2, 32, 35, 36], except for *FGF2*, *CD44*, *FAS*, *BAX*, and *BCL2* which were designed with the PrimerQuest Tool, and validated by gel electrophoresis. Relative expression values for each target gene were calculated using the ΔΔCt method with efficiency correction and using one control sample as calibrator [37], which was different for each experiment. The housekeeping gene was *CYCA* as previously validated in our laboratory [2].

Statistical analysis

Meiosis progression, maturation, cumulus expansion, and apoptosis data were arcsine transformed before analysis, and gene expression and matrix viscosity data were transformed to logarithms when not normally distributed. The effects of treatments were tested by analysis of variance (ANOVA), and means were compared with the Fisher-protected test, using JMP software (SAS Institute, Cary, NC, USA). Differences were considered significant when $P < 0.05$.

Table 1 Information of specific primer used for amplification in real-time PCR

Genes	Primer sequence	Fragment size (bp)	Annealing temperature (°C)	Reference
CYC-A	F: 5'-GCCATGGAGCGCTTTGG-3' R: 5'-CCACAGTCAGCAATGGTGATCT-3'	65	60	Machado et al., 2009
FGF2	F: 5'-GCAAACCGTTACCTTGCTATG-3' R: 5'-ACCACCTGGAGTATTTCCTTGA-3'	136	60	NM_174056.4
NPPC	F: 5'-TCAGCCTCCTCGCATCT-3' R: 5'-ACAGCTGGTGTTGTGTATCC-3'	101	60	de Lima et al., 2016
NPR2	F: 5'-ATGACAGCATCAACCTGGACTGGA-3' R: 5'-AGCACGAAACGACTATCCACCACA-3'	145	60	de Lima et al., 2016
HAS2	F: 5'-ACACAGACAGGCTGAGGACAACTT-3' R: 5'-AAGCAGCTGTGATTCCAAGGAGGA-3'	133	60	Caixeta et al., 2013
CD44	F: 5'-GACCCTCAATCTTCCTCTTCAC-3' R: 5'-TTTCCTACCAGGTGCCAATC-3'	94	60	NM_174013.3
PTGS2	F: 5'-AAGCCTAGCACTTTCGGTGGAGAA-3' R: 5'-TCCAGAGTGGGAAGAGCTTGCATT-3'	168	60	Caixeta et al., 2013
PTX3	F: 5'-CCTCAGCTATCGGTCCATAA-3' R: 5'-ATTGAAGCCTGTGAGGTCTGC-3'	294	54	Caixeta et al., 2009
TNFAIP6	F: 5'-GCAAAGGAGTGTGGTGGTGTGTTT-3' R: 5'-ACTGAGGTGAATGCGCTGACCATA-3'	135	60	Caixeta et al., 2013
VCAN	F: 5'-TTTGAGAACCAGACAGGCTTCCCT-3' R: 5'-TTGGTGTCAATTCTGTCCCAGTCCCA-3'	172	60	Machado et al., 2015
FAS	F: 5'-GCAACTCTGCAGCCTCAAATG-3' R: 5'-CATCATTTTGGCTTCCTCCATACC-3'	153	59	NM_174662.2
nPR	F: 5'-AGAACTCATCAAGGCAATTGG-3' R: 5'-CACATCCCTGCCAATATCTTG-3'	108	60	Machado et al., 2015
BAX	F: 5'-TGTTTTCTGACGGCAACTTCA-3' R: 5'-CGAAGGAAGTCCAATGTCCAG-3'	139	60	NM_173894.1
BCL2	F: 5'-TTCGCCGAGATGTCCAGTCAGC-3' R: 5'-TTGACGCTCTCCACACACATGACC-3'	155	60	NM_001166486.1

Results

Abundance of *FGF2* mRNA in cumulus cells transiently increased during IVM, reaching maximal values at 8 h, then returning to levels not different from those observed in immature COC at 20 h of IVM (Fig. 1(A)). At 12 h of IVM, FSH increased *FGF2* mRNA levels in a dose-dependent manner (Fig. 1(B)). Removal of the oocyte by oocytectomy did not significantly affect *FGF2* mRNA abundance at 4 h of IVM, but significantly decreased it at 22 h, which was restored by the addition of denuded oocytes to the culture medium (Fig. 2).

Addition of FGF2 to the IVM medium increased the percentage of oocytes reaching GVBD at 6 h only at the lowest

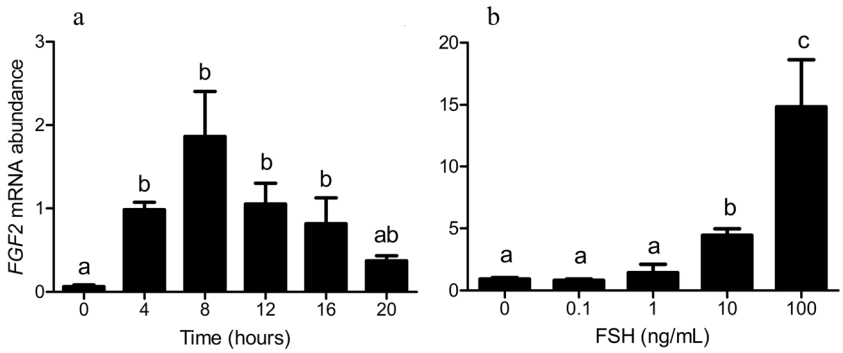

Fig. 1 Effects of time in culture (A; FSH at 10 ng/mL, equivalent to 2×10^{-5} UI/mL); and FSH graded doses (B) on relative values of *FGF2* mRNA abundance in bovine cumulus cells undergoing IVM. Data are presented as mean values (± S.E.M.) relative to a calibrator sample calculated by the ΔΔCt method with efficiency correction. Different calibrator samples were utilized in the time-course (A) and dose-response (B) experiments. Bars with different letters are significantly different ($P < 0.05$)

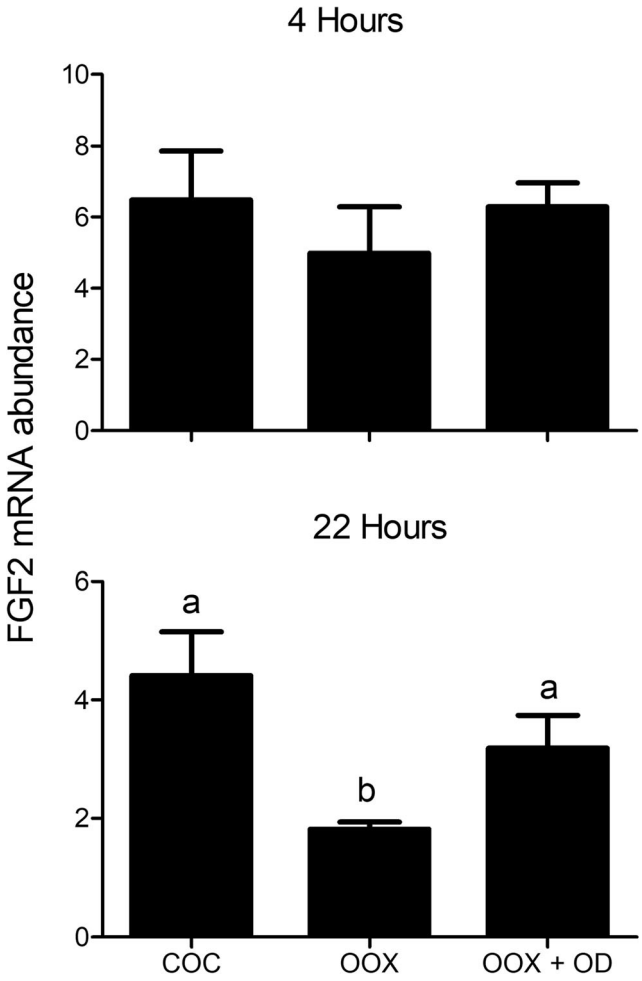

Fig. 2 Effects of oocyte removal on relative values of *FGF2* mRNA expression. Intact (COC), oocytectomized (OOX), and OOX plus denuded oocytes (OOX + OD; 1 oocyte/μL) were subjected to IVM for 4 h ($n = 4$ replicates) and 22 h ($n = 5$ replicates). Data are presented as mean values (± S.E.M.) relative to a calibrator sample calculated by the ΔΔCt method with efficiency correction. Different calibrator samples were utilized in experiments assessing the effect of oocyte removal at 4 and 22 h of IVM. Bars with different letters are significantly different ($P < 0.05$)

dose tested (1 ng/mL; Fig. 3(A)) but did not affect the percentage of oocytes reaching meiosis II at 22 h of IVM at any of the doses tested (Fig. 3(B)). These data suggest that FGF2 accelerates oocyte maturation. Abundance of mRNA of genes expressed in cumulus cells that regulate GVBD (*NPPC* and *NPR2*) was not affected by FGF2.

Supplementation of the IVM medium with FGF2 did not alter the percentage of COC exhibiting full expansion, but decreased dissociation of cumulus cells (Fig. 4). Regarding effects on genes regulating cumulus expansion, FGF2 decreased abundance of *PTGS2* mRNA at all concentrations tested and of *TNFAIP6* mRNA at the highest dose (100 ng/mL). Abundance of *HAS2*, *CD44*, *PTX3*, and *VCAN* mRNA was not significantly affected by FGF2 (Fig. 5).

Treatment with FGF2 increased the percentage of viable non-apoptotic cumulus cells at 100 ng/mL and reduced the percentage of cumulus cells with caspases 3/7 activated at 10 and 100 ng/mL, while it did not alter the proportion of cells staining for annexin V at the end of IVM (Fig. 6). FGF2 did not affect mRNA abundance of genes regulating apoptosis (*FAS*, *BAX*, *BCL2*, or *nPR*).

Discussion

Previous studies have shown that expression of *FGF2* increases after the LH surge in bovine cumulus cells, suggesting a role for FGF2 in the mechanisms underlying peri-ovulatory COC maturation [21]. In the present study, we demonstrate that in an IVM setting, FSH also increases *FGF2* mRNA levels in bovine cumulus cells. More interestingly, the present data demonstrate regulation of *FGF2* mRNA levels in cumulus cells by factors secreted by the oocyte and provide evidence that FGF2 regulates cumulus matrix formation, oocyte nuclear maturation, and cumulus cell survival.

The potential role of FGF2 in vivo remains unclear, although exogenous FGF2 can improve developmental competence of bovine and porcine oocytes during IVM [29, 30]. We show here that addition of low doses of FGF2 to COC undergoing IVM enhanced the rate of GVBD at 6 h of IVM,

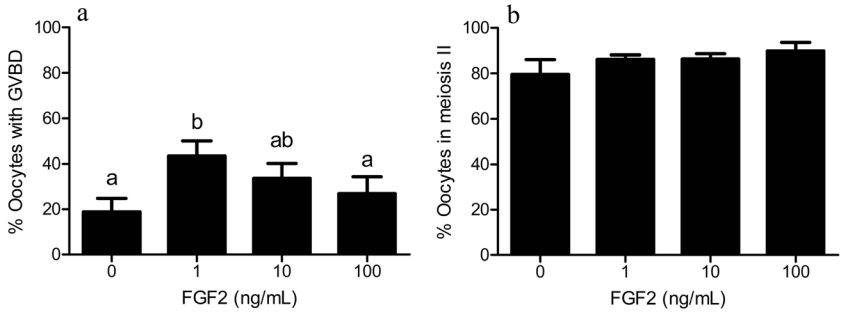

Fig. 3 Effects of FGF2 on progression of meiosis. COC were subjected to IVM with graded doses of FGF2 for 6 h to assess germinal vesicle status (A; GVBD = germinal vesicle breakdown; $n = 5$ replicates) and for 22 h to assess meiosis II completion (B; MII = meiosis II/includes oocytes from telophase I to metaphase II; $n = 5$ replicates). Bars with different letters are significantly different ($P < 0.05$)

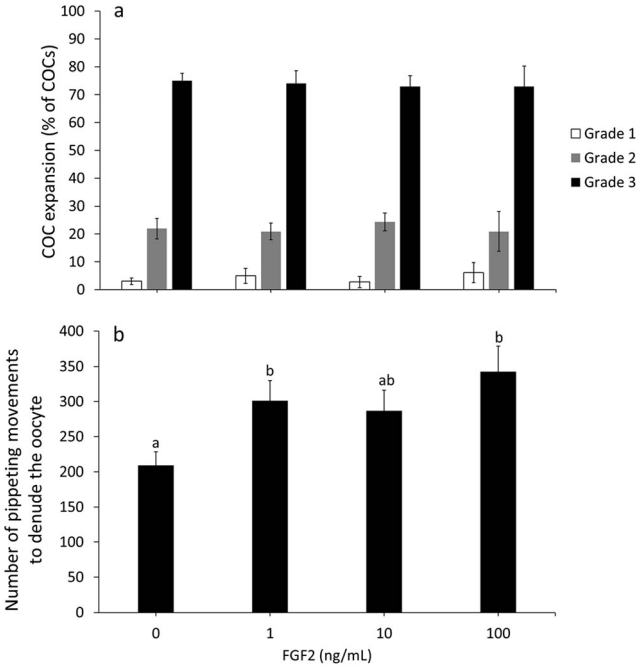

Fig. 4 Effects of FGF2 on the percentage of COC exhibiting different degrees of cumulus expansion (A; Grade 1: fully expanded, Grade 2: partially expanded, Grade 3: weakly or not expanded; $n = 5$ replicates) and on dissociation of cumulus cells (B; $n = 5$ replicates). Bars with different letters are significantly different ($P < 0.05$)

although it did not alter metaphase II rates at the end of IVM. Since LH activity inhibits expression of *NPPC* in murine granulosa cells [38], and upregulates *FGF2* expression in bovine cumulus cells [21], we hypothesized that FGF2 could be a mediator of the effects of LH on meiosis progression by decreasing *NPPC/NPR2* expression. However, FGF2 did not alter mRNA abundance of *NPPC* and *NPR2* in bovine cumulus cells in the present study. Alternatively, the advance in GVBD caused by FGF2 during IVM is likely a direct consequence of enhanced ERK1/2 activation. We have previously demonstrated that FGF2 increases phosphorylation of ERK1/2 in bovine granulosa cells [39], and this signaling pathway has been proposed as the major trigger of meiosis resumption [4].

Although the degree of cumulus expansion was not affected, we observed that the quality of the cumulus mass was altered by FGF2, as it became more difficult to dissociate cumulus cells and to remove the expanded cumulus from the oocyte. It is known that the cumulus ECM is different during in vivo and in vitro maturation [40] and a 'suboptimal' cumulus ECM is associated with lower developmental competence of the oocyte [20]. Therefore, the capacity of FGF2 to regulate matrix quality may be related to its ability to improve the rate of blastocyst formation when added to the IVM medium [29–31].

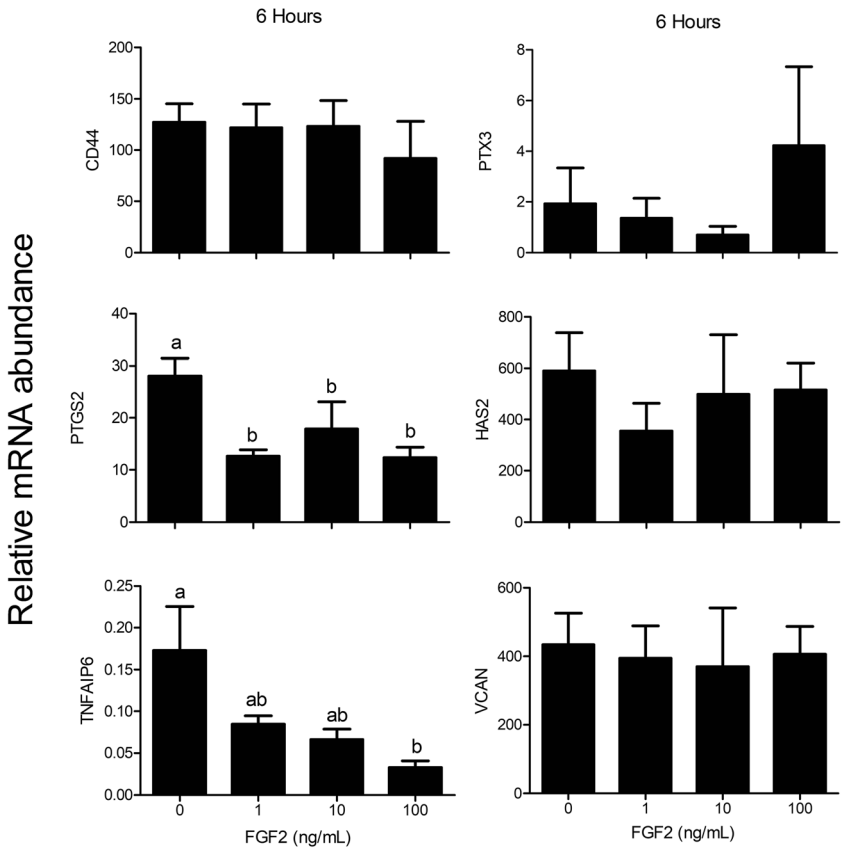

Fig. 5 Effects of FGF2 on mRNA abundance of genes regulating cumulus expansion. COC were subjected to IVM with graded doses of FGF2 for 6 h ($n = 5$ replicates). Data are presented as mean values (± S.E.M.) relative to a calibrator sample by the ΔΔCt method with efficiency correction. Bars with different letters are significantly different ($P < 0.05$)

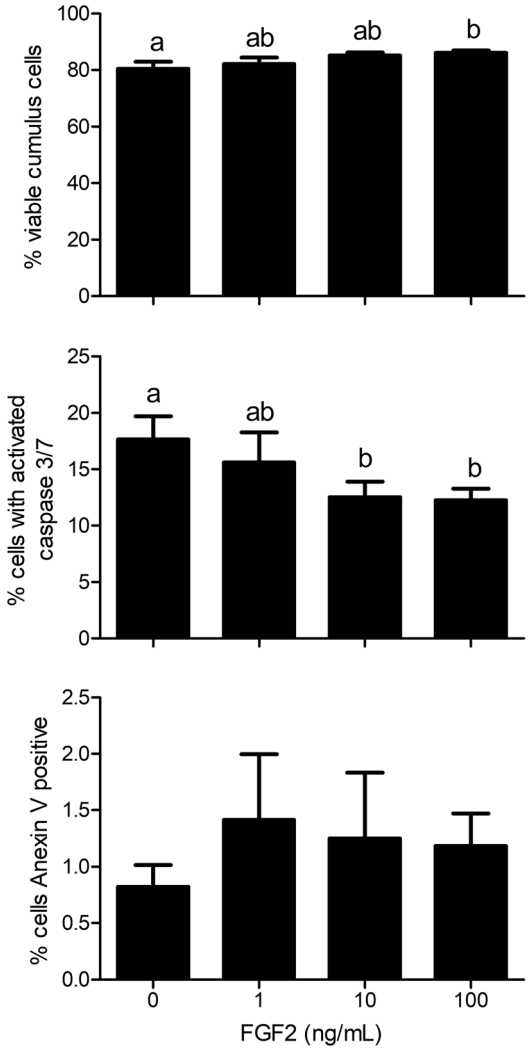

Fig. 6 Effects of FGF2 on the percentage of viable cumulus cells (cells marked with hoechst 33342 but not with propidium iodide), of cumulus cells with activated caspase 3/7, and of cumulus cells stained for Annexin V ($n = 6$ replicates). Data are presented as means (± S.E.M.) and bars with different letters are significantly different ($P < 0.05$)

The interaction of PTX3 with TSG6, the protein encoded by *TNFAIP6*, regulates ECM stability during cumulus expansion [8]. As both are cross-linking proteins that attach to HA via HCs of IαI [41], it is logical to assume that PTX3/TSG6 interaction regulates ECM resistance and elasticity. Therefore, it is fair to speculate that the FGF2-induced decrease in *TNFAIP6* expression could restrict TSG6 availability in the ECM thus altering the ease with which cumulus cells could be dissociated in the present study. Interestingly, a direct physical interference of FGF2 in ECM organization is also possible since FGF2 can bind to both PTX3 and TGS6. In fact, previous studies have demonstrated that TGS6 and PTX3 compete to bind FGF2, which constitutes an important mechanism in the local control of FGF2 angiogenic activity [42, 43]. Therefore, FGF2 may directly decrease PTX3/TSG6 interaction also in the ECM of the COC thus regulating its physical characteristics.

The inhibition of *PTGS2* mRNA abundance by FGF2 was not expected, as it is known to stimulate *PTGS2* mRNA abundance in the corpus luteum and other tissues [44, 45]. Although *HAS2* is partly controlled by prostaglandins [28, 46], FGF2 did not alter *HAS2* mRNA abundance, which agrees with the absence of an impact on the degree of cumulus expansion in the present study. On the other hand, the suppressive effect of FGF2 on *PTGS2 mRNA* is consistent with the decrease in *TNFAIP6* mRNA levels since prostaglandin signaling stimulates *TNFAIP6* expression [47]. It is thus possible that the degree at which *PTGS2* expression was suppressed by FGF2 could impact on *TNFA6* expression, but not on *HAS2*. Interestingly, the effects of FGF2 reported herein contrast with positive effects of oocyte derived FGF10 on the degree of cumulus expansion and *PTGS2* mRNA abundance [33, 48]. This discrepancy is likely a consequence of differential receptor affinity; while FGF2 preferably activates FGFR2C and FGFR3C, FGF10 activates more efficiently FGFR1B and FGFR2B [49]. Therefore, it appears that different FGFs from different cell sources can differently regulate ECM formation in the COC.

Addition of FGF2 to the IVM medium also decreased the proportion of apoptotic cumulus cells as measured by activated caspase-3/7 and staining by propidium iodide after 22 h IVM. This is consistent with previously reported effects of FGF2 on granulosa cells [26, 27], and suggests that increased FGF2 signaling before and during the ovulatory process helps to maintain healthy cumulus cells. As discussed below, the present study demonstrates that oocyte-secreted factors can increase *FGF2* expression in the COC. This observation coupled with the pro-survival effect of FGF2 on cumulus cells suggests that FGF2 may be an important mediator in the previously reported anti-apoptotic influence of oocyte-secreted factors on cumulus cells [50].

The transient increase in *FGF2* mRNA abundance during IVM observed in *Bos indicus* (present study) is very similar to that previously observed in *Bos taurus* cows [24]. Further, we demonstrate here regulation of *FGF2* mRNA levels in cumulus cells by FSH. However, gonadotropins are not likely the sole regulators as removal of the oocyte resulted in a decrease in *FGF2* mRNA abundance, and replacement with denuded oocytes restored *FGF2* mRNA levels. This suggests that oocyte-secreted factors contribute to the control of cumulus gene expression in cattle, as has been demonstrated also for *KITL* and *NPPC* mRNA levels [32]. The observation that oocyte-secreted factors increased *FGF2* mRNA levels at 22 but not at 4 h of IVM suggests that, although it does not appear to represent an acute regulatory loop, it occurs within a time window compatible with the exposure of cumulus cells to OSFs during follicle development. Together with previous studies demonstrating upregulation of *FGF2* expression after the LH surge in vivo [21, 22], the present data reinforce that transcription of *FGF2* is enhanced in preparation for ovulation in bovine cumulus cells, while providing novel evidence that

the oocyte plays a role in the control of *FGF2* expression in cumulus cells via secreted factors.

Conclusion

In summary, *FGF2* expression is enhanced at the time the ovulatory cascade is activated to participate in the control of different processes relevant to developmental competence. We present novel data indicating that FGF2 expression is regulated by oocyte-secreted factors, and that FGF2 production would then favor meiosis resumption, cumulus expansion quality, and cumulus cell survival. Therefore, this study has practical implications for IVM/IVF while contributing for the understanding of FGF2 physiological roles as a mediator in the ovulatory cascade.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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