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Fibroblast growth factor 17 and bone morphogenetic protein 15 enhance cumulus expansion and improve quality of *in vitro* produced embryos in cattle.

Short Title: FGF17 & BMP15 stimulate cumulus expansion and improve embryo development

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ABSTRACT

Bone morphogenetic protein 15 (BMP15) and members of the fibroblast growth factor (FGF) family are expressed by the oocyte and are involved in the control of cumulus cell function. We tested the hypothesis that FGF17, alone or combined with BMP15 in the maturation medium, enhances cumulus expansion, meiosis progression, embryonic development and expression of mRNA encoding key genes regulating expansion [prostaglandin-endoperoxide synthase 2 (*PTGS2*), hyaluronan synthase 2 (*HAS2*), tumor necrosis factor-stimulated gene 6 (*TNFAIP6*) and pentraxin 3 (*PTX3*)] and markers of oocyte developmental competence [phosphofructokinase (*PFKP*), gremlin (*GREM1*), versican (*VCAN*) and the genomic progesterone receptor (*nPR*)] in cumulus cells. FGF17 and BMP15 increased the percentage of fully expanded cumulus-oocyte complexes (COCs) but there was not additive effect when both were combined. Neither FGF17 nor BMP15 altered the percentage of oocytes reaching meiosis II at the end of COC culture, or cleavage and blastocyst rates following IVF. However, embryo quality, as assessed by the number of cells in the inner cell mass, was improved by the combination of FGF17 with BMP15. FGF17 alone did not alter gene expression in cumulus cells at the end of *in vitro* maturation (IVM), whereas BMP15 increased *PTGS2* and *PTX3* mRNA levels. The combination of FGF17 and BMP15 increased *nPR* mRNA abundance in cumulus cells but did not change the expression of other markers of developmental competence. This study provides novel evidence that FGF17 enhances cumulus expansion in bovine COCs submitted to IVM, and that the supplementation of the IVM medium with FGF17 and BMP15 may improve embryo quality.

Keywords: FGF17, BMP15, oocyte maturation, cumulus expansion, embryo quality.

1. INTRODUCTION

Communication between the oocyte and cumulus cells is bidirectional and essential for maturation of the cumulus-oocyte complex (COC) and generation of an embryo with high potential for development. The oocyte regulates differentiation of cumulus cells through the secretion of oocyte secreted factors (OSF), such as bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9), both of which are members of the transforming growth factor β (TGFβ) superfamily [1]

Cumulus expansion is a critical event in the periovulatory process that culminates in resumption of meiosis and ovulation. Expansion is triggered by the LH surge, which causes the release of the EGF-like ligands; amphiregulin (AREG) and epiregulin (EREG) and betacellulin (BTC) from granulosa cells [2]. These proteins then activate the EGF receptor on cumulus cells to induce expression of EREG, AREG, BTC and prostaglandin-endoperoxide synthase 2 (PTGS2). Increased PTGS2 activity increases prostaglandin secretion and increases expression of hyaluronan synthase 2 (HAS2), tumor necrosis factor-stimulated gene 6 (TNFAIP6) and pentraxin 3 (PTX3), necessary for expansion of the COC [3, 4] .In mice, TGFβ superfamily members, particularly BMP15 and GDF9, promote cumulus expansion and it has been proposed that, together, they regulate expansion under physiological conditions [1]. BMP15 has been shown to enhance cumulus expansion in mice and cattle [5-7], and to enhance bovine oocyte oxidative metabolism via cumulus cell mediated mechanisms [8, 9]. It has also become apparent that BMP15 acts in concert with other growth factors, as BMP15-stimulated cumulus expansion was enhanced by the addition of GDF9 in the mouse [10]. Interestingly, whereas BMP15 and GDF9 separately only weakly affected abundance of

mRNA encoding natriuretic peptide receptor B (NPR2) in murine cumulus cells, together they induced higher expression, at levels comparable to those promoted by the oocyte [11]. Another oocyte-derived protein is fibroblast growth factor 8 (FGF8), and this also acts as a partner for BMP15 to stimulate NPR2 expression [11]. GDF9 and FGF8 are not redundant cofactors for BMP15, as BMP15 plus FGF8 increased glycolytic activity in mouse cumulus cells whereas BMP15 plus GDF9 did not [6].

FGF8 is the prototype member of a FGF subfamily that also contains FGF17 [12]. FGF17 protein was localized to the oocyte of preantral and antral follicles in the bovine ovary [13], and activates receptors (FGFR2c, FGFR3c and FGFR4) that are expressed in granulosa cells, cumulus cells and oocytes [7, 14]. Little is known about the function of FGF17 in the ovary, although FGF17 inhibited steroidogenesis in bovine granulosa cells [13]. As FGF17 and FGF8 are structurally similar and activate the same receptors, it would be interesting to see if FGF17 also regulates the differentiation of cumulus cells.

The objective of this study was to test the hypothesis that FGF17, alone or in combination with BMP15, enhances cumulus cell function and COC developmental competence. Specifically, we determined whether FGF17 alone or in combination with BMP15 alters expansion, meiosis progression, embryonic development and the abundance of mRNA encoding key genes regulating expansion *(PTGS2*, *HAS2*, *PTX3* and *TNFAIP6*) and markers of oocyte developmental competence in cumulus cells [phosphofructokinase (*PFKP*), gremlin (*GREM1*), versican (*VCAN*), the genomic progesterone receptor (*nPR*); [15-17].

2. MATERIALS AND METHODS

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

2.1. *In vitro* maturation

Ovaries of adult cows (predominantly Nellore, *Bos indicus*) were obtained at an abattoir local to the Sao Paulo State University campus in Botucatu and transported to the laboratory in saline solution (0.9% NaCl) containing antibiotics (penicillin G; 100 IU/ml and streptomycin; 100 ug/ml) at 35-37°C. COCs were aspirated from 3 to 8 mm diameter follicles with an 18 gauge needle and pooled in a 15 ml conical tube. After sedimentation, COCs were recovered and selected using a stereomicroscope. Only COCs with homogeneous cytoplasm and compact multilayer of cumulus cells were used (Grade 1 and 2). COCs were washed and transferred in groups of 20 to a 200 µl drop of maturation medium, TCM199 containing Earle's salts supplemented with 1 ug/ml porcine FSH (equivalent to 0.002 IU; Folltropin-V® Bioniche Animal Health, Belleville ON, Canada), 10 IU/ml LH (Lutropin-V ®, Bioniche Animal Health), 22 ug/ml sodium pyruvate, 75 ug/ml amicacin, 4 mg/ml BSA and growth factors (see below). Drops were covered with mineral oil and incubated at 38.5°C in 5% CO_2 in humidified air.

2.2. Cumulus expansion assessment

The effect of graded doses of recombinant human FGF17 (R&D Systems, Minneapolis, MN, USA; 0, 1, 10 and 100 ng/ml; n=4) on cumulus expansion were tested after 22 hours of culture. To test potential synergism between BMP15 and FGF17 in the regulation of cumulus expansion, an additional experiment was performed with a minimally effective dose [7] of recombinant human BMP15 alone (R&D Systems, Minneapolis, MN, USA; 100 ng/ml; n=4), FGF17 alone (100 ng/ml; n=4) and BMP15 (100 ng/ml) plus FGF17

(100 ng/ml; n=4). Cumulus expansion was visually assessed at 22 hours of culture according to a subjective scoring system. Grades 1 to 3 were attributed to increasing degrees of expansion (1-poor expansion, characterized by few morphological changes compared with before maturation; 2-partial expansion, characterized by fair expansion but notable clusters lacking expansion; 3-complete or nearly complete expansion;[14].

2.3. Meiosis progression following oocyte maturation

The effects of BMP15 (100 ng/ml), FGF17 (100 ng/ml) and FGF17+BMP15 (100 ng/ml each) on meiosis progression was assessed after 22 hours of maturation. Oocytes were denuded by pipetting in PBS fixed with 4% paraformaldehyde (Polysciences, Inc., Warrington, PA, USA) and stained for 15 min with Hoechst 33342 (1mg/mL Invitrogen H-1399). Chromatin status and meiotic staging of oocytes was determined by epifluorescence microscopy as meiosis arrested at metaphase I (MI), meiotically mature (telophase I and metaphase II) or degenerate (Deg), as described previously [18, 19]. Data were derived from four independent replicates with 20 oocytes.

2.4. Gene expression analysis

To test the effects of BMP15 (100 ng/ml) and FGF17 (100 ng/ml) alone and in combination on gene expression, maturation medium was supplemented with growth factors as described above. After 22 hours of culture, cumulus cells and oocytes were mechanically separated by repeated pipetting in PBS. Cumulus cells were transferred to 1.5 ml tubes, washed twice by centrifugation for 5 min at 700g, and 350 µl of RNA extraction lysis buffer from RNeasy® kit (Qiagen, Mississauga, ON, Canada) was added to the cell pellets. Samples were stored at -80°C until RNA extraction.

Total RNA was extracted from cumulus cells 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. Total RNA concentrations were 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/µg; Invitrogen, São Paulo, Brazil) and then reverse transcribed using Oligo-dT primers and Omniscript reverse transcriptase (Qiagen, Mississauga, ON, CA). The reagents were incubated at 37ºC for 60 min and then at 93º for 3 min for enzyme inactivation.

Relative real time RT-PCR analysis was performed with an ABI 7500 thermocycler using Power Sybr Green PCR Master Mix (Applied Biosystems, São 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 sec followed by annealing for 1 min (40 cycles). The primers sequences, amplicons sizes and annealing temperatures for GREM1, VCAN and nPR are shown in Table 1 and all other primers are given in Caixeta et al. 2013. Reactions were optimized to provide maximum amplification efficiency for each gene. The specificity of the PCR products was assessed by melting curve analyses and amplicon size determined by electrophoresis in 2% agarose gels.

Cyclophilin-A (*CYCA*) was selected as the housekeeping gene [7].The relative expression values for each gene were calculated using the ΔΔCt method with efficiency correction and using one control sample as calibrator [20], which was determined from the amplification profile of individual samples with LinRegPCR software [21]. Each sample was run in duplicate.

2.5. *In vitro* embryo production

To determine the effect of FGF17, BMP15 or both proteins during oocyte in vitro maturation and subsequent embryo development, ten immature oocytes were cultured in 100 µl drops of maturation medium, TCM199 supplemented with 25 mM sodium bicarbonate, 0.46 mM sodium pyruvate, 4 mg/ml BSA (free of fatty acids; ICP Biological, Auckland, New Zealand) and 0.1 IU/ml FSH (Puregon, Organon, Oss, Netherlands), and FGF17 (100 ng/ml; R&D Systems), or BMP15 (100 ng/ml, R&D Systems), or FGF17+BMP15 (each 100 ng/ml; R&D Systems) overlayered with mineral oil for 23-24 hours.

Post IVM, mature oocytes were fertilized with a final concentration of $1x10^6$ spermatozoa/ml. The presumptive zygotes were cultured for 5 days in VitroCleave® (IVF Vet Solutions, Adelaide, Australia). On day 5, embryos were transferred into VitroBlast® (IVF Vet Solutions) for another 3 days. The blastocysts were assessed on days 7 and day 8. Expanded blastocysts, hatching blastocysts and hatched blastocysts were fixed and stained on day 8.

2.6. Differential staining

Differential staining was performed using a modified method described by. Expanded, hatching and hatched blastocysts were placed into 1% (v/v) Triton X-100 containing 100 µg/ml propium iodide for 20-30 seconds or until the trophectoderm visibly changed colour and shrunk. Blastocysts were washed in absolute ethanol before incubation in 25 µg/ml Hoechst 33342 solution in ethanol. Blastocysts were whole mounted in a drop of glycerol on microscope slides and covered with cover slip. The differential staining was examined under a fluorescence microscope (Olympus, Tokyo, Japan) at 200x equipped

with an ultraviolet dichroic mirror. The inner cell mass (ICM) stained blue and trophectoderm (TE) nuclei stained pink.

2.7. Statistical Analysis

Cumulus expansion data were arcsine transformed before analysis, and gene expression data were transformed to logarithms when not normally distributed. The effects of treatments with BMP15 and/or FGF17 on cumulus cell expansion and gene expression were tested by analysis of variance (ANOVA), and means were compared with the Tukey-Kramer HSD test using JMP software (SAS Institute, Cary, NC, USA). Data for embryo development were arcsine transformed and blastocyst cell number were log transformed before analysis with one way ANOVA with least significant difference (LSD) post hoc using SigmaStat software (SPSS Inc, Chicago, IL, USA) The results are presented as means ± standard error of the mean (SEM). Differences were considered significant when $P<0.05$.

3. RESULTS

FGF17 and BMP15 on cumulus expansion and meiosis progression

COCs were exposed to graded doses of FGF17 and cumulus expansion was recorded. The percentage of grade 3 COCs (complete or nearly complete cumulus expansion) was increased by FGF17 at 100 ng/ml and the same dose of FGF17 decreased the percentage of grade 1 COCs (no expansion; Fig. 1A). To test for interactions between FGF17 and BMP15, COCs were cultured with FGF17, BMP15 or the combination of both proteins. BMP15 and FGF17 each enhanced cumulus expansion compared to controls and no

additive or synergistic effect was observed when both proteins were added to the maturation media compared to either alone (Fig. 1B).

To determine whether supplementation with FGF17, BMP15 or the combination of both proteins affects oocyte maturation, COCs were denuded after 22 h of maturation and meiotic staging was assessed. The percentage of oocytes reaching telophase I or metaphase II (TI/MII) after 22 h of maturation was not influenced by either protein (Fig. 2). Supplementation with FGF17 and BMP15 tended to increase the percentage of TI/MII oocytes, but was not statistically different. Interestingly, no degenerated oocytes were observed when COCs were treated with FGF17 plus BMP15 during IVM.

Effects of FGF17 and BMP15 on embryo development

Supplementation with FGF17 and/or BMP15 during oocyte maturation did not alter cleavage and embryo development rates, nor the percentage of hatching blastocysts at day 8 post IVF (Fig. 3).

Differential staining was performed on a subset of blastocysts (Table 2) to investigate whether supplementation with FGF17 and/or BMP15 during oocyte maturation affects embryo quality as assessed by the number of cells in the inner cell mass (ICM) and trophectoderm. There was a significant increase in the number of cells in the inner cell mass in the FGF17+BMP15 group compared to the control group (Table 2).

Effects of FGF17 and/or BMP15 on cumulus gene expression

To gain possible insights into the mechanisms by which FGF17 enhanced cumulus expansion, we examined whether FGF17 regulates expression of key genes involved in

the ovulatory cascade. FGF17 did not affect abundance of mRNA encoding *HAS2*, *PTX3*, *PTGS2* and *TNFAIP6* (Fig. 4) at the end of maturation.

BMP15 alone increased *PTGS2* and *PTX3* mRNA levels (Fig. 5). FGF17 and BMP15 in combination stimulated *nPR* mRNA expression, which was not observed when the proteins were added separately (Fig. 5). FGF17 and BMP15 had no effect on *HAS2*, *PFKP*, *GREM1* and *VERS* mRNA abundance at 22 hours of maturation (Fig. 5).

4. DISCUSSION

In the mouse, FGF8 and BMP15 cooperate to metabolic activity of cumulus cells, but neither are effective alone, which has led to the concept that BMP and FGF pathways interact for the full expression of oocyte developmental competence in mice [6]. In the present study, we assessed whether a closely related growth factor, FGF17, acts alone or with BMP15 to improve expansion and developmental competence of cow oocytes. The important findings of this study are that FGF17 is effective alone in improving cumulus expansion, and cooperates with BMP15 to improve developmental competence of the oocyte.

In mice, FGF8 is an oocyte-specific factor that acts in concert with BMP15 to increase glycolytic activity of cumulus cells, however FGF8 appears to play no role in the process of cumulus expansion [10]. In mice and cattle, FGF17 is also expressed in the oocyte and activates the same receptors as FGF8 [7, 12, 23]. Intriguingly, unlike previous reports with FGF8 in mice [10], FGF17 increased COC expansion in cattle in the present study. An unrelated FGF, FGF10, altered glucose metabolism favouring hyaluronic acid production and stimulated expansion of cumulus cells [7] and developmental competence

in cattle [14], suggesting there are species and/or ligand-specific differences in FGF actions in the COC.

The mechanism by which FGFs affect expansion is not clear. The ability of BMP15 to do so in mice and cattle has been associated with increased levels of mRNA encoding EGF-like ligands as well as the downstream proteins PTGS2, PTX3 and TGS6 [5, 7]. FGF10 appears to act at a more distal point in the preovulatory cascade, as it stimulated *PTGS2, PTX3* and *TGS6* mRNA levels without altering the upstream EGF-like ligands [7]. In the present study, FGF17 stimulated cumulus expansion without an increase in levels of mRNA encoding *PTGS2*, *PTX3* and *TNFAIP6;* the possibility remains that FGF17 acts at the protein/enzyme activity level and not at the transcriptional level.

Although neither FGF17 nor BMP15 alone increased developmental competence in the present study, when combined they increased abundance of mRNA encoding the progesterone receptor in cumulus cells and the number of cells in the inner cell mass of day 8 blastocysts. A larger inner cell mass is associated with increased embryo and/or fetal viability in mice, humans and cattle [24-26], and bovine blastocysts with a smaller ICM were found to have an increased rate of apopotosis [26]. Progesterone is antiapoptotic in cumulus cells [27] and blocking progesterone receptor activity with RU486 decreased embryo development in cattle [28]. Therefore, while speculative, the present data suggest that FGF17 increases *nPR* expression, which in turn may lead to less apoptotic cumulus cells and increased developmental competence. In addition, the association of embryo quality with *nPR* mRNA abundance in the present study is consistent with the identification of this gene as a potential marker of developmental competence in bovine cumulus cells [29].

These data are consistent with previous reports showing that native oocyte-secreted factors are potent stimulators of developmental competence in cattle and mice as well as other species [24, 30]. The use of recombinant oocyte-secreted factors in IVM appears to be complicated by the form of protein used. When used alone, the isolated mature region homodimer of BMP15 (as supplied by R&D Systems) is ineffective at enhancing competence in mouse IVM [24] and has no or modest effects in bovine IVM [31]; current study, whereas the BMP15 homodimer pro-mature complex is effective in bovine IVM [24, 30, 31]. Interestingly in the current study, whilst the mature region BMP15 alone did not increase competence, it was effective in combination with FGF17, suggesting the need for cooperation of additional signalling pathways, facilitated by FGFs, for oocyte developmental competence.

Considering previous data in mice showing that FGF8, the prototype member of the FGF subfamily containing FGF17 synergizes with BMP15 to stimulate glycolytic activity and *PFKP* mRNA expression [6], we investigated whether the positive effect of the combination of FGF17+BMP15 on embryo quality was associated with an increase in mRNA levels of the rate limiting glycolytic enzyme PFKP. However, the combination of FGF17 with BMP5 did not alter *PFKP* mRNA abundance. Apart from reflecting potential species-specific differences or different intracellular pathways activated by these structurally related FGFs, these results may also reflect differences in the culture systems utilized; the most obvious being the use of intact COCs in the present study and oocytectomized COCs in the mouse study. We cannot rule out the participation of members of the FGF8 subfamily in the physiological regulation of glycolysis in the bovine COC.

In summary, the present data suggest that FGF17 stimulates cumulus expansion in bovine COCs submitted to IVM through mechanisms different to those previously reported for other oocyte secreted factors. Moreover, FGF17 together with BMP15 improves blastocyst quality indicating this combination may be useful to improve the outcomes of IVM/IVF protocols.

DECLARATION OF INTEREST

The University of Adelaide owns a patent family on the application of BMP15 to IVM and RBG and JGT are inventors. Both RBG and JGT have received research funding from Cook Medical Pty Ltd for BMP15 research, but not used for the research described here. The remaining 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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REFERENCES

[1] Gilchrist RB, Lane M, Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Hum Reprod Update. 2008;14:159-77.
[2] Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science. 2004;303:682-4.
[3] Conti M, Hsieh M, Park JY, Su YQ. Role of the epidermal growth factor network in ovarian follicles. Mol Endocrinol. 2006;20:715-23.
[4] Shimada M, Hernandez-Gonzalez I, Gonzalez-Robayna I, Richards JS. Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: key roles for prostaglandin synthase 2 and progesterone receptor. Mol Endocrinol. 2006;20:1352-65.
[5] Yoshino O, McMahon HE, Sharma S, Shimasaki S. A unique preovulatory expression pattern plays a key role in the physiological functions of BMP-15 in the mouse. Proc Natl Acad Sci U S A. 2006;103:10678-83.
[6] Sugiura K, Su YQ, Diaz FJ, Pangas SA, Sharma S, Wigglesworth K, et al. Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. Development. 2007;134:2593-603.
[7] Caixeta ES, Sutton-McDowall ML, Gilchrist RB, Thompson JG, Price CA, Machado MF, et al. Bone morphogenetic protein 15 and fibroblast growth factor 10 enhance cumulus expansion, glucose uptake, and expression of genes in the ovulatory cascade during in vitro maturation of bovine cumulus-oocyte complexes. Reproduction. 2013;146:27-35.
[8] Sutton-McDowall ML, Mottershead DG, Gardner DK, Gilchrist RB, Thompson JG. Metabolic differences in bovine cumulus-oocyte complexes matured in vitro in the presence or absence of follicle-stimulating hormone and bone morphogenetic protein 15. Biol Reprod. 2012;87:87.
[9] Sugimura S, Ritter LJ, Sutton-McDowall ML, Mottershead DG, Thompson JG, Gilchrist RB. Amphiregulin co-operates with bone morphogenetic protein 15 to increase bovine oocyte developmental competence: effects on gap junction-mediated metabolite supply. Mol Hum Reprod. 2014;20:499-513.
[10] Sugiura K, Su YQ, Li Q, Wigglesworth K, Matzuk MM, Eppig JJ. Estrogen promotes the development of mouse cumulus cells in coordination with oocyte-derived GDF9 and BMP15. Mol Endocrinol. 2010;24:2303-14.
[11] Zhang M, Su YQ, Sugiura K, Xia G, Eppig JJ. Granulosa cell ligand NPPC and its receptor NPR2 maintain meiotic arrest in mouse oocytes. Science. 2010;330:366-9.
[12] Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. Trends Genet. 2004;20:563-9.
[13] Machado MF, Portela VM, Price CA, Costa IB, Ripamonte P, Amorim RL, et al. Regulation and action of fibroblast growth factor 17 in bovine follicles. J Endocrinol. 2009;202:347-53.
[14] Zhang K, Hansen PJ, Ealy AD. Fibroblast growth factor 10 enhances bovine oocyte maturation and developmental competence in vitro. Reproduction. 2010;140:815-26.
[15] Li Q, McKenzie LJ, Matzuk MM. Revisiting oocyte-somatic cell interactions: in search of novel intrafollicular predictors and regulators of oocyte developmental competence. Mol Hum Reprod. 2008;14:673-8.

[16] Adriaenssens T, Segers I, Wathlet S, Smitz J. The cumulus cell gene expression profile of oocytes with different nuclear maturity and potential for blastocyst formation. J Assist Reprod Genet. 2011;28:31-40.
[17] Gebhardt KM, Feil DK, Dunning KR, Lane M, Russell DL. Human cumulus cell gene expression as a biomarker of pregnancy outcome after single embryo transfer. Fertil Steril. 2011;96:47-52.e2.
[18] Roth Z, Hansen PJ. Disruption of nuclear maturation and rearrangement of cytoskeletal elements in bovine oocytes exposed to heat shock during maturation. Reproduction. 2005;129:235-44.
[19] Marei WF, Wathes DC, Fouladi-Nashta AA. The effect of linolenic Acid on bovine oocyte maturation and development. Biol Reprod. 2009;81:1064-72.
[20] Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45.
[21] Ramakers C, Ruijter JM, Deprez RH, Moorman AF. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett. 2003;339:62-6.
[22] Thouas GA, Korfiatis NA, French AJ, Jones GM, Trounson AO. Simplified technique for differential staining of inner cell mass and trophectoderm cells of mouse and bovine blastocysts. Reprod Biomed Online. 2001;3:25-9.
[23] Zhang X, Ibrahimi OA, Olsen SK, Umemori H, Mohammadi M, Ornitz DM. Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. J Biol Chem. 2006;281:15694-700.
[24] Sudiman J, Ritter LJ, Feil DK, Wang X, Chan K, Mottershead DG, et al. Effects of differing oocyte-secreted factors during mouse in vitro maturation on subsequent embryo and fetal development. J Assist Reprod Genet. 2014;31:295-306.
[25] Lane M, Gardner DK. Differential regulation of mouse embryo development and viability by amino acids. J Reprod Fertil. 1997;109:153-64.
[26] Knijn HM, Gjørret JO, Vos PL, Hendriksen PJ, van der Weijden BC, Maddox-Hyttel P, et al. Consequences of in vivo development and subsequent culture on apoptosis, cell number, and blastocyst formation in bovine embryos. Biol Reprod. 2003;69:1371-8.
[27] Salhab M, Tosca L, Cabau C, Papillier P, Perreau C, Dupont J, et al. Kinetics of gene expression and signaling in bovine cumulus cells throughout IVM in different mediums in relation to oocyte developmental competence, cumulus apoptosis and progesterone secretion. Theriogenology. 2011;75:90-104.
[28] Aparicio IM, Garcia-Herreros M, O'Shea LC, Hensey C, Lonergan P, Fair T. Expression, regulation, and function of progesterone receptors in bovine cumulus oocyte complexes during in vitro maturation. Biol Reprod. 2011;84:910-21.
[29] Assidi M, Dufort I, Ali A, Hamel M, Algriany O, Dielemann S, et al. Identification of potential markers of oocyte competence expressed in bovine cumulus cells matured with follicle-stimulating hormone and/or phorbol myristate acetate in vitro. Biol Reprod. 2008;79:209-22.
[30] Hussein TS, Thompson JG, Gilchrist RB. Oocyte-secreted factors enhance oocyte developmental competence. Dev Biol. 2006;296:514-21.
[31] Sudiman J, Sutton-McDowall ML, Ritter LJ, White MA, Mottershead DG, Thompson JG, et al. Bone morphogenetic protein 15 in the pro-mature complex form enhances bovine oocyte developmental competence. PLoS One. 2014;9:e103563.