Effect of kit ligand on natriuretic peptide precursor C and oocyte maturation in cattle

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Abstract

In vitro maturation (IVM) of oocytes in cattle is inefficient, and there is great interest in the development of approaches to improve maturation and fertilization rates. Intraovarian signalling molecules are being explored as potential additives to IVM media. One such factor is kit ligand (KITL), which stimulates the growth of oocytes. We determined if KITL enhances oocyte maturation in cattle. The two main isoforms of KITL (KITL1 and KITL2) were expressed in bovine cumulus–oocyte complexes (COC), and levels of mRNA increased during FSH-stimulated IVM. The addition of KITL to the culture medium increased the percentage of oocytes that reached meiosis II but did not affect cumulus expansion after 22h of IVM. Addition of KITL reduced the levels of mRNA encoding natriuretic peptide precursor C (NPPC), a protein that holds oocytes in meiotic arrest, and increased the levels of mRNA encoding YBX2, an oocyte-specific factor involved in meiosis. Removal of the oocyte from the COC resulted in increased KITL mRNA levels and decreased NPPC mRNA levels in cumulus cells, and addition of denuded oocytes reversed these effects. Taken together, our results suggest that KITL enhances bovine oocyte nuclear maturation through a mechanism that involves NPPC, and that the oocyte regulates cumulus expression of KITL mRNA.

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Introduction

Ovulation is induced by the luteinizing hormone (LH) surge, which causes the increased expression of epidermal growth factor (EGF)-like proteins in granulosa and cumulus cells, and subsequent induction of prostaglandin-endoperoxide synthase 2 (PTGS2) mRNA/protein and prostaglandin secretion. Prostaglandins induce the expansion of the cumulus complex and proteolytic degradation of the follicle wall, and are involved in oocyte nuclear maturation (Richards 2005, Reizel et al. 2010, Prochazka et al. 2012, Marei et al. 2014).

Before the LH surge, oocytes are held in meiotic arrest by high intraoocyte levels of cAMP that are maintained at least in part by the transfer of cGMP from cumulus cells to the oocyte through gap junctions (Shuhaibar et al. 2015). The generation of cGMP is driven by natriuretic peptide precursor C (NPPC), which is secreted from granulosa and cumulus cells and activates natriuretic peptide receptor B (NPR2) on cumulus cells (Zhang et al. 2010a, Franciosi et al. 2014, De Cesaro et al. 2015). Loss of NPPC expression results in resumption of meiosis (Zhang et al. 2010a, Kawamura et al. 2011, Robinson et al. 2012) and addition of NPPC to cumulus–oocyte complexes (COC) in vitro delayed resumption (Zhang et al. 2010a, Franciosi et al. 2014). During follicle growth, NPPC levels are high, and are decreased by hCG in preovulatory follicles (Liu et al. 2014). Thus, growing follicles produce quantities of NPPC sufficient to prevent premature resumption of meiosis, and the preovulatory LH surge relieves this brake on oocyte maturation.

Cytoplasmic maturation must be tightly synchronized with nuclear maturation for the oocyte to achieve developmental competence. Accumulation of mRNA stocks that allow protein production until the embryo becomes transcriptionally competent is a crucial component of cytoplasmic maturation since transcriptional activity progressively decreases as chromatin condensation advances in the germline vesicle (Hyttel et al. 1986, Lodde et al. 2007). Y-box binding protein 2 (YBX2) appears to be a key regulator in this context; YBX2 increases mRNA content, mRNA stability and protein synthesis in mouse oocytes.
(Yu et al. 2004, Medvedev et al. 2011). In the bovine oocyte, YBX2 expression decreases as maturation progresses from the germinal vesicle stage to metaphase II (Vigneault et al. 2009).

Another factor involved in oocyte maturation is kit ligand (KITL), which is secreted from granulosa cells and acts on receptors (KIT) on the oocyte (Hutt et al. 2006, Thomas & Vanderhyden 2006, Celestino et al. 2010). Alternative splicing of KITL mRNA results in a full-length and a truncated protein, termed KITL1 and KITL2 respectively, and both are biologically active (Brannman et al. 1991, Huang et al. 1992, Zhou et al. 1994, Kawaguchi et al. 2007). Although KITL is well known to stimulate growth and survival of oocytes (Packer et al. 1994, Ismail et al. 1997, Thomas et al. 2008), its effects on nuclear maturation are controversial: addition of KITL to denuded rat oocytes reduced the rate of the 1st polar body extrusion after 14 h culture (Ismail et al. 1996, 1997), whereas it increased the rate of the 1st polar body extrusion after 22 h in mice (Ye et al. 2009).

It is well established that the oocyte regulates cumulus cell function in mice, but the role the oocyte plays in cattle is less clear. The oocyte is required for cumulus glycolysis and expansion in mice (Eppeg 2005, Sugiuara et al. 2005, Vanderhyden 1993) but not in cattle (Ralph et al. 1995, Sutton et al. 2003). Removal of the oocyte from the COC (oocytectomy) in mice reduced the levels of mRNA encoding certain genes in cumulus cells including Nppc (Lee et al. 2013), and increased those of other genes including Kitl (Joyce et al. 1999); a microarray study with bovine oocytectomized cumulus cells did not identify NPPC or KITL as genes affected by the oocyte (Regassa et al. 2011). The inhibitory effect of the murine oocyte on cumulus cell Kitl mRNA levels is likely mediated through the secretion of growth/differentiation factor 9 (Gdf9) (Joyce et al. 2000), although another oocyte-secreted factor, bone morphogenetic factor (Bmp) 15, has been shown to stimulate cumulus Kitl mRNA levels in rodents (Otsuka & Shimasaki 2002, Thomas et al. 2005, Miyoshi et al. 2012).

It is not known if the bovine oocyte regulates KITL or NPPC expression in cattle, and although NPPC has been shown to delay meiotic resumption in cattle (Franciosi et al. 2014), the impact of KITL on meiotic resumption in cattle has not been reported. In this study, we tested the hypothesis that KITL signaling regulates COC maturation under the influence of the oocyte in the cow. The specific objectives were to determine the regulation of KITL expression by the oocyte and putative oocyte-secreted factors, and to measure the effects of KITL on cumulus expansion, NPPC mRNA levels and oocyte maturation.

Materials and methods

Unless specified, all chemicals and reagents were purchased from Sigma.

Experimental design

The patterns of expression of KITL1 and KITL2 mRNA in cumulus cells and of KIT mRNA in oocytes were evaluated during IVM at 0, 4, 8, 12, 16 and 22 h (N=4/time). To investigate the effects of oocyte-secreted factors on the abundance of KITL1 mRNA in cumulus cells, the culture medium was supplemented with graded doses of recombinant BMP15 (N=4/dose), FGF8 (N=5/dose) or FGF17 (N=4/dose; R&D Systems). The effect of KITL on COC maturation was determined by culturing COC for 22 h with 0, 10, 50 or 100 ng/mL recombinant KITL1 (R&D Systems) to evaluate cumulus expansion and abundance of mRNA encoding key proteins involved in expansion (N=4/dose), and the progression of the oocyte through meiosis (N=9/dose) as well as key genes involved in nuclear maturation (N=4/dose). The effect of the oocyte and oocyte-secreted factors on the expression of KITL and NPPC in cumulus cells was tested by the comparison of mRNA levels from intact COCs, oocytectomized COCs and oocytectomized COCs cultured with denuded oocytes (1 denuded oocyte/μL; N=4/group).

In vitro maturation

Ovaries of adult cows (predominantly Nellore, Bos indicus) were obtained at an abattoir local to the São Paulo State University campus in Botucatu and transported to the laboratory in saline solution (0.9% NaCl) at 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 100 μL drop of maturation medium, TCM199 containing Earle’s salts supplemented with 1 μg/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 μg/mL sodium pyruvate, 75 μg/mL amicacin, 4 mg/mL BSA and growth factors. Drops were covered with mineral oil and incubated at 38.5°C in 5% CO2 in humidified air.

Oocytectomy

Oocyte–cumulus complexes 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 described (Buccione et al. 1990) with modifications (Paradis et al. 2010).

Assessment of cumulus expansion

Cumulus expansion was visually assessed at 22 h of culture according to a subjective scoring system. Grades 1–3 were attributed to increasing degrees of expansion (Grade 1: poor expansion, characterized by few morphological changes compared with before maturation; Grade 2: partial expansion, characterized by fair expansion but notable clusters lacking expansion; Grade 3: complete or nearly complete expansion (Machado et al. 2015).
Meiosis progression: oocyte nuclear maturation

Meiosis progression was assessed after 22 h of maturation. Oocytes were denuded by pipetting in PBS and stained for 20 min with Hoechst 33342 (1 mg/mL, Invitrogen H-1399) under a UV excitation using epifluorescence microscopy, and stage of meiosis were determined and classified as metaphase I (MI) or telophase I/metaphase II (TI/MII).

Gene expression analysis

After culture, cumulus cells and oocytes from 20 COCs were mechanically separated by repeated pipetting in PBS without calcium and magnesium. Cumulus cells were transferred to 1.5 mL tubes, collected by centrifugation for 5 min at 700 g and frozen at −80 °C in 350 µL of RNA extraction lysis buffer from the RNeasy kit (Qiagen). Total RNA was extracted using the RNeasy kit (Qiagen). Total RNA was extracted using oligo-dT primers and Omniscript (for cumulus cells) or DNAse I (1 U/µL) for oocytes) reverse transcriptases (Qiagen). The analysis was performed with an ABI 7500 thermocycler using Sensiscript (for oocytes) reverse transcriptases (Qiagen). The reagents were incubated at 37 °C for 60 min and then at 93 °C for 3 min for enzyme inactivation. Relative real-time RT-PCR analysis was performed with PrimerQuest Tool and are shown in Table 1. The relative expression values for each gene were calculated using the ΔΔCt method with efficiency correction and using one control sample as calibrator (Pfaffl 2001). Cyclophilin-A was used as the housekeeping gene as previously validated in our laboratory (Caixeta et al. 2013a).

Statistical analysis

Maturation and 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 growth factors on cumulus cell expansion, maturation 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). Differences were considered significant when P < 0.05.

Results

KITL1, KITL2 and KIT mRNA levels in COCs during IVM

To determine whether KITL undergoes alternative splicing in cattle, primers were designed to span the potential splice junction, and PCR performed on cumulus cell RNA. Agarose gel electrophoresis revealed two bands, and sequencing identified one product lacking 84 nucleotides of exon 3 that corresponds to KITL2, and another product including exon 3 corresponding to KITL1. Subsequent real-time PCR was performed with primers specific to each splice variant. Average Ct values for KITL1 and KITL2 mRNA in cumulus cells were 28.8 and 29.3 cycles respectively. Relative abundance of KITL1 and KITL2 mRNA increased during IVM with

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Fragment size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
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<tr>
<td>BMP1</td>
<td>F: 5′GTACGACAGCCAGAGGTAGTG3′</td>
<td>360</td>
<td>59</td>
<td>Caixeta et al. (2013a)</td>
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<tr>
<td></td>
<td>R: 5′CCCGAGGACATCTCCCTC3′</td>
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<td>GDF9</td>
<td>F: 5′TGTTCTTTCTGAAGACATCTAG3′</td>
<td>202</td>
<td>59</td>
<td>Caixeta et al. (2013a)</td>
</tr>
<tr>
<td></td>
<td>R: 5′ACAGTGAGAGCTAGGTGCTCTC3′</td>
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<tr>
<td>KIT</td>
<td>F: 5′ATCATGAACCATCTGCTGATC3′</td>
<td>128</td>
<td>60</td>
<td>AF263827.1</td>
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<td></td>
<td>R: 5′GGGCTCAGTGTTGTAAGTGGA3′</td>
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<tr>
<td>YBX2</td>
<td>F: 5′TGTCCTGCAATCCACAGT3′</td>
<td>118</td>
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<td></td>
<td>R: 5′CTTCTTCTCCCTCAGACATC3′</td>
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<tr>
<td>KL1</td>
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<td>KL2</td>
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<td>R: 5′AGCAAACCCGATCACAAGA3′</td>
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<td>CCNB1</td>
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<td>PTCS2</td>
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<td>168</td>
<td>60</td>
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<td>R: 5′TCAGCTGGGAAAGCTGCTC3′</td>
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<tr>
<td>CYC-A</td>
<td>F: 5′GCCATGGACGCCCTTTGG3′</td>
<td>65</td>
<td>60</td>
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<tr>
<td></td>
<td>R: 5′CACCAGTCGACATGGTATC3′</td>
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</table>

F, forward primer; R, reverse primer.
significantly greater levels at 12 h compared with 0–8 h (Fig. 1). The abundance of KIT mRNA in the oocyte did not vary during IVM.

**Effect of KITL on cumulus expansion and oocyte maturation**

Addition of recombinant KITL1 to IVM medium did not affect cumulus expansion, but significantly stimulated the progression of meiosis as indicated by the increased proportion of oocytes reaching meiosis II (Fig. 2). Furthermore, KITL1 increased the levels of mRNA encoding the germ cell-specific marker YBX2, but did not alter those encoding CCNB1, BMP15 and GDF9 in the oocyte, or NPR2 in cumulus cells (Fig. 3).

Addition of KITL1 decreased the abundance of NPPC mRNA in cumulus cell and had no effect on cumulus cell NPR2 or PTGS2 mRNA levels.

**Effects of the oocyte or oocyte-secreted factors on KITL mRNA levels**

Removal of the oocyte significantly increased the abundance of mRNA encoding KITL1 and KITL2 in cumulus

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**Figure 1** Abundance of KITL1, KITL2 (A) and KIT (B) mRNA in cumulus cells during IVM. Groups of immature COCs represent 0h. Messenger RNA abundance was measured by real-time PCR. Data are presented as mean values (±S.E.M.) relative to a calibrator sample and were calculated by the ΔΔCt method with efficiency correction. Bars with different letters are significantly different (a, b, c for KITL1/x, y, z for KITL2; P < 0.05). Data were derived from four independent replicates.

**Figure 2** Effect of KITL1 on meiosis progression (A) and cumulus expansion (B) after 22 h of IVM. Oocytes were classified as MI (meiosis I; A1) or MI/MII (oocytes in the transition telophase I/metaphase II; A2). The degree of cumulus expansion was classified as grades 1 (poor expansion), 2 (partial expansion) and 3 (full expansion). Different letters within meiosis stages indicate significant differences (P < 0.05).

**Figure 3** Effect of KITL1 on mRNA levels of genes regulating COC maturation in cumulus cells (NPPC, NPR2 and PTGS2) and in the oocyte (CCNB1, YBX2, BMP15 and GDF9). Messenger RNA abundance was measured by real-time PCR. 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). Data were derived from four independent replicates.
Kit ligand affects oocyte maturation and NPPC

cells, and addition of denuded oocytes reversed this effect (Fig. 4). In the same samples, oocytectomy significantly decreased NPPC mRNA levels, which again was restored to control levels by coculture with denuded oocytes (Fig. 4).

Figure 4 Effects of oocyte removal and oocyte replacement on KITL1, KITL2 and NPPC mRNA levels in cumulus cells. COCs were cultured intact (COC), oocytectomized (OOX) or oocytectomized with denuded oocytes (OOX + DO) for 22 h. Messenger RNA abundance was measured by real-time PCR. 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). Data were derived from four independent replicates. Illustrative photomicrographs of culture groups before and after IVM (A and D: intact COCs; B and E: OOX; C and F: OOX plus denuded oocytes), and of the oocytectomy procedure (G).

The addition of FGF8 to intact COC in IVM medium for 22 h increased cumulus cell KITL1 and KITL2 but decreased NPPC mRNA levels, addition of BMP15 increased only KITL1 mRNA levels, addition of FGF10

Figure 5 Effects of putative oocyte-secreted factors on KITL1, KITL2 and NPPC mRNA levels in cumulus cells. COCs were cultured with graded doses of FGF8, FGF10, BMP15 and GDF9 for 22 h of IVM. Messenger RNA abundance was measured by real-time PCR. 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). Data were derived from four independent replicates.
in intact COCs, suggesting the role of an oocyte-secreted factor (OSF). A previous study demonstrated the role of the bovine oocyte in maintaining cumulus cell health (Hussein et al. 2005); collectively, these data point to a role for the oocyte in cumulus function in cattle, but one that differs from that in the mouse.

In rodents, Gdf9 is an abundant OSF (Crawford & McNatty 2012) and inhibits Kitl mRNA levels in cumulus cells (Joyce et al. 2000). The murine oocyte may therefore maintain low Kitl expression levels through the secretion of Gdf9 (Joyce et al. 2000, Otsuka and Shimasaki 2002, Thomas et al. 2005, Miyoshi et al. 2012), and thus removal of the oocyte increased Kitl mRNA levels (Joyce et al. 1999). In the cow, however, it is believed that BMP15 is the more abundant OSF compared with GDF9 (Crawford & McNatty 2012) and so the oocyte may not control cumulus Kitl mRNA in the same manner. Moreover, a recent study points to a physiological and more effective action for the premature form of the heterodimer BMP15-GDF9 named cumulin in relation to BMP15 and GDF9 homodimers in monovular species (Mottershead et al. 2015). In this study, FGF8 and BMP15 stimulated Kitl mRNA levels, as in the mouse, and FGF10 inhibited Kitl mRNA abundance. Therefore, the increase in Kitl mRNA levels following oocytectomy in cattle may be at least in part attributable to the loss of FGF10, and not to the influence of oocyte-derived FGF8 and BMP15 homodimer. The reduction in Nppc mRNA levels induced by FGF8 is likely mediated by Kitl signalling given the stimulatory effect of FGF8 on Kitl expression and the inhibitory effect of Kitl on Nppc expression. In contrast, FGF10 appears to inhibit Nppc expression by a different mechanism since it decreased Kitl2 mRNA levels without affecting those of Kitl1. The decrease in Nppc expression induced by FGF10 is consistent with its previously reported stimulatory effect on oocyte nuclear maturation (Zhang et al. 2010b).

In this study, both Kitl1 and Kitl2 mRNA levels increased with time during cumulus expansion. This is consistent with the increase in Kitl mRNA levels observed in granulosa and cumulus cells in mice in response to eCG (Ismail et al. 1996, Ye et al. 2009). The effects of Kitl on oocyte maturation are not yet clear. Addition of Kitl to denuded rat oocytes reduced the rate of the 1st polar body extrusion after 14 h culture (Ismail et al. 1996), whereas it increased the rate of the 1st polar body extrusion after 22 h in mice (Ye et al. 2009) and the rate of oocytes reaching MII in cattle (present study). The importance of this effect is not clear, as in mice, treatment with Kitl did not enhance the rate of blastocyst development (Ye et al. 2009). The Kitl-induced increase in rates of maturation in the mouse was associated with increased levels of Ccnb1 protein in MII oocytes (Ye et al. 2009), whereas in this study, Kitl did not alter Ccnb1 mRNA levels in MII oocytes. As Ccnb1 is involved in meiosis 1, it is likely that the time point chosen in this study was too late to detect an effect of Kitl.

**Discussion**

In mice, the oocyte is critical for several cumulus cell functions including expansion, and regulates the levels of mRNA encoding Nppc and Kitl, two proteins involved in resumption of meiosis. In cattle, the oocyte is not necessary for expansion, and effects of the oocyte on cumulus cell NPPC and KITL mRNA levels have not previously been reported. In this study, we demonstrate that, surprisingly, the bovine oocyte regulates these two genes in a manner very similar to that previously observed in the mouse, and we show that Kitl enhances nuclear maturation in the cow. We also link Kitl and NPPC for the first time, demonstrating that Kitl downregulates NPPC mRNA levels in cumulus cells, and suggesting that this is a mechanism by which Kitl increases the maturation of the oocyte. A model depicting the paracrine interaction involving Kitl, NPPC, YBX2 and oocyte-derived factors in the bovine COC is proposed (Fig. 6).

One of the more interesting results of this study is the similarity in the response of cumulus cell Kitl and NPPC mRNA levels to oocytectomy observed here in cattle with that previously observed in mice (Joyce et al. 1999, Lee et al. 2013). This appears to be unusual in that cumulus function in cattle, such as metabolism and expansion (Ralph et al. 1995, Sutton et al. 2003) and appears not to be impacted by the removal of the oocyte. Equally novel is the ability of denuded bovine oocytes to completely restore Kitl and NPPC mRNA levels to those observed
Addition of KITL to COC decreased the abundance of mRNA encoding NPPC, and this may offer an explanation for the mechanism of action of KITL. Resumption of meiosis is triggered by a decrease in cAMP levels in the oocyte, which is the result of decreased transfer of cAMP and cGMP from cumulus cells (Norris et al 2009). There is no evidence that KITL alters cumulus cGMP levels, and KITL did not alter FSH-stimulated cAMP levels in rat granulosa–oocyte cocultures (Miyoshi et al 2012). However, NPPC has been demonstrated to increase cumulus cell cGMP levels and maintain meiotic arrest in rodents and cattle (Zhang et al 2010a, Franciosi et al 2014); therefore, the decrease in NPPC mRNA caused by KITL observed here might be expected to lead to a decrease in NPPC signalling and of cGMP levels, and thus the resumption of meiosis. We are not aware of any other reports demonstrating the regulation of NPPC levels by KITL. In the present dataset, although the highest dose of KITL promoted the maximal decrease in NPPC mRNA abundance, it did not alter oocyte nuclear maturation. A possible explanation for this intriguing observation is that high doses of KITL may precociously and intensively suppress NPPC/NPR2 signalling leading to abrupt closure of gap junctions, which could be detrimental to meiosis resumption.

A further novel finding of this study is the stimulatory effect of KITL on oocyte YBX2 mRNA levels. In this study, we used oocytes from follicles 3–8mm in diameter, which are predominantly at advanced stages of the germinal vesicle and with low transcriptional activity (Lodde et al 2007, 2008), and oligo-dT primers in the RT-PCR strategy. Therefore, this result more likely reflects an increase in adenylated YBX2 mRNA. In female mice, Ybx2 is expressed exclusively in the oocyte and encodes a very abundant protein that stabilizes maternal mRNA, sustains protein synthesis and is required for normal spindle formation (Yu et al 2001, 2002, 2004, Medvedev et al 2011). Mice null for Ybx2 (Yang et al 2005) and adult mice are characterized by degeneration and loss of oocytes. Interestingly, in juvenile Ybx2+/− mice, with a less pronounced loss of oocytes, follicles were unable to ovulate in response to exogenous gonadotrophin (Yang et al 2005), suggesting that Ybx2 may play some role in oocyte signalling to the cumulus cells and the mechanism of ovulation. In cattle, YBX2 protein levels decrease from the germinal vesicle stage to meiosis II (Vigneault et al 2009). Although speculative, the present data suggest that KITL may enhance YBX2 action in the bovine oocyte, contributing for RNA stability and protein production during the maternal-embryo transition and for normal spindle formation/meiosis completion.

In summary, the present data show that KITL mRNA levels increase during IVM in cattle, and that addition of KITL1 to COC enhances resumption of meiosis. Two potential mechanisms by which KITL may increase oocyte maturation are by inhibiting the expression of NPPC, a factor that maintains meiotic arrest, and by increasing expression of YBX2, an oocyte-specific protein essential for ovulation and oocyte health. In addition, we provide evidence that the oocyte regulates the expression of KITL and NPPC in cumulus cells, suggesting a novel mechanism by which the oocyte may influence its own fate.

Declaration 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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