Exocannabinoids effect on in vitro bovine oocyte maturation via activation of AKT and ERK1/2

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Abstract

Endocannabinoids are known to mediate practically all reproductive events in mammals; however, little is known about their role in oocyte maturation. Through RT-PCR and immunocytochemistry, this study confirms the presence of CB1 and CB2 cannabinoid receptors in bovine oocytes and shows how exposure to the exogenous cannabinoids HU-210 and THC during their in vitro maturation (IVM) activates the phosphorylation of AKT and ERK1/2 proteins associated with the resumption of meiosis. Although supplementation with HU-210 or THC during IVM did not increase blastocyst yields, the expression of interferon tau (IFNτ) and gap junction alpha-1 protein (GJA1) was enhanced at the blastocyst stage. Our data suggest that cannabinoid agonists may be useful IVM supplements as their presence during oocyte maturation upregulates the expression in blastocysts of key genes for embryo quality.

Introduction

The molecular mechanisms responsible for oocyte maturation are not fully understood and various molecules seem capable of modulating this process. Among these molecules, an important role of the cannabinoid compounds, fatty acid derivatives that exert their effects by binding to membrane cannabinoid receptor 1 (CNR1 or CB1) and cannabinoid receptor 2 (CNR2 or CB2), both Gi/o protein-coupled receptors, has been proposed (Devane et al. 1988, Matsuda et al. 1990). Many of the components of the endocannabinoid system have been identified in the ovary, oocyte, granulosa cells and embryos of mice, rats and humans (Wang et al. 2004, El-Talatini et al. 2009a, Peralta et al. 2011, López-Cardona et al. 2014, Agirregoitia et al. 2015, Miller et al. 2016). In effect, the best characterized endocannabinoid, anandamide (AEA), is present in human follicular fluid and its concentrations rise during oocyte maturation (Schuel et al. 2002, Wang et al. 2003, El-Talatini et al. 2009b). A recent study in cattle has identified AEA in oviductal fluid with highest concentrations found during the periovulatory period (Gervasi et al. 2013). However, the presence of AEA receptors on the oocyte has not yet been reported. In addition, cell signalling exerted by cannabinoids and the signalling that occurs when oocyte meiosis resumes both in humans (Peralta et al. 2011) and mice (Paria et al. 1995, Schmitt & Nebreda 2002) share several similar features.

In human clinical practice, immature oocytes harvested from antral ovarian follicles can be in vitro matured (IVM) in culture media supplemented with different molecules to promote maturation. Despite disappointing low embryo production and pregnancy rates offered by this technique (Barnes et al. 1995, Barnes et al. 1996, Cha et al. 2000), IVM could be the only option for women intolerant to gonadotropins (Cha et al. 2000) wishing to undergo in vitro fertilization (IVF). Before any clinical trial is designed to improve IVM, research is needed in an animal model similar to the human model such as cow (Menezo & Herubel 2002). Humans and cows are single ovulators and show close similarity in ovarian function and oocyte characteristics (Van Hoeck et al. 2011). Indeed, bovine oocyte maturation shares features with the human model in terms of ovarian follicular dynamics and endocrine control (Menezo & Herubel 2002, Campbell et al. 2003). Some authors even proposed the bovine maturation model as a reliable surrogate method to screen for toxic agents or metabolic studies in human oocytes (Van Hoeck et al. 2011, Beker van Woudenberg et al. 2012, Santos et al. 2014).
Despite this useful model, investigations to date examining the role of endocannabinoids in bovine reproduction events have mainly focused on the sperm physiology and sperm–oviduct interaction. These efforts have revealed that AEA induces sperm capacitation through CB1 and the transient receptor potential vanilloid 1 (TRPV1) activation, and modulates sperm release from the oviductal reservoir (Gervasi et al. 2011, 2016). Moreover, recently it has been demonstrated that endocannabinoid signalling governs sperm activation via sex hormone progesterone (Miller et al. 2016), a non-genomic signal that is also required for oocyte maturation (Revelli et al. 1998). Based on the above discussion, the aim of this study is to confirm the presence of cannabinoid receptors in bovine oocytes and whether the presence of exogenous cannabinoids, such as HU-210 ((6aR,10aR)-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol) or THC (Δ9-tetrahydrocannabinol), in the maturation medium activates pathways involved in oocyte maturation like protein kinase B (also known as AKT) and extracellular signal-regulated kinase (ERK1/2) phosphorylation and thus improves in vitro blastocyst quality without compromising embryo development.

Materials and methods

Unless otherwise stated, all chemicals and media were purchased from Sigma Chemical Co.

Isolation and in vitro maturation of cumulus–oocyte complexes

Immature cumulus–oocyte complexes (COCs) were obtained by aspirating follicles (2–8 mm) from the ovaries of matured heifers and cows collected at slaughter from a local abattoir. COCs (homogenous cytoplasm and intact cumulus cells) were matured in four-well dishes (Nunc, Roskilde, Denmark) in 500 µL TCM-199 maturation medium, supplemented with 10% (v/v) foetal calf serum (FCS) and 10 ng/mL epidermal growth factor (EGF) in groups of 50 COCs per well for 24 h at 38.5°C in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂, with maximum humidity. Cleavage rate was recorded on day 2 (48 h post insemination) and cumulative blastocyst yield was recorded on days 7, 8 and 9 post insemination.

In vitro culture

Presumptive zygotes were denuded by gentle vortexing for 3 min, washed twice in phosphate buffer saline (PBS) supplemented with 3% BSA and cultured in groups of 25 in 25 µL droplets of synthetic oviduct fluid (SOF) (Holm et al. 1999) supplemented with 5% FCS under mineral oil at 38.5°C in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂, with maximum humidity. Embryo development was scored according to the criteria of Khatir et al. (1998). Embryo development and blastocyst rate were recorded on days 7, 8 and 9 post insemination.

IVM supplementation with cannabinoid agonists

COCs were co-incubated with the cannabinoid agonists HU-210 and THC (concentration of 100 nM in 0.1% DMSO) in maturation medium, according to prior optimization experiments (López-Cardona et al. 2014). COCs incubated with and without DMSO served as controls. To determine the impact of HU-210 and THC on oocyte maturation, COCs were matured in four-well dishes (Nunc, Roskilde, Denmark) in 500 µL TCM-199 maturation medium, supplemented with 5% FCS under mineral oil at 38.5°C in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂, with maximum humidity. Cleavage rate was recorded on day 2 (48 h post insemination) and cumulative blastocyst yield was recorded on days 7, 8 and 9 post insemination.

Meiotic progression of bovine oocytes undergoing IVM with exogenous cannabinoids

In order to determine the impact of HU-210 and THC on meiotic progression of bovine oocytes, maturation experiments were performed on COCs from both experimental groups and control with DMSO at 2, 4, 6, 8 and 12 h of IVM (n = 30 per time point and group in three independent replicates) were used as described previously (Khatir et al. 1998). Briefly, COCs were partially denuded by vortexing during 3 min in 0.1% hyaluronidase (Sigma H3506) and fixed in 4% paraformaldehyde (Panreac, Barcelona, Spain) for 20 min. Then were washed twice in PBS and fixed in PBS containing 10 µg/mL Hoechst 33342 for 15 min. Oocytes were then placed in glass slides and squashed with coverslip in order to visualize the nuclear stage under microscopy (Nikon Optiphot-2) (Coy et al. 2008).

Immunofluorescence

Pools of 20 COCs were obtained from DMSO group for the different maturation stages: The first group was immediately removed from the follicle representing the GV stage and incubated in PBS containing 100 µM H3506 and fixed in 4% paraformaldehyde (Panreac, Barcelona, Spain) for 20 min. Then were washed twice in PBS and fixed in PBS containing 10 µg/mL Hoechst 3342 for 15 min. Oocytes were then placed in glass slides and squashed with coverslip in order to visualize the nuclear stage under microscopy (Nikon Optiphot-2) (Coy et al. 2008).
room temperature. After permeabilization, oocytes were incubated overnight at 4°C in PBS containing 1% PVA, 5% FCS and 1:100 rabbit polyclonal anti-cannabinoid CB1 receptor (Cayman Chemical) and anti-cannabinoid CB2 receptor (Cayman Chemical) antibodies. Following incubation, oocytes were washed twice in PBS with 1% PVA and incubated in PBS supplemented with 5% FCS and 1:250 goat polyclonal secondary antibody Alexa Fluor 488 (Molecular Probes) for 2 h at room temperature followed by washing again three times in PBS – 1% PVA. In all cases, nuclei were stained with Hoechst 33342 at a concentration of 0.01 mg/mL during the second wash to determine the maturation stage of each oocyte (Gutierrez-Adan & Pintado 2000). Finally, oocytes were mounted in microprecipets with Fluoromount G (EMS, Hatfield, UK) and examined by confocal microscopy (Leyca TCS-SPE). Negative controls were prepared using blocking peptide and omitting the primary antibody before adding the secondary antibody. To monitor AKT and ERK1/2 activation during oocyte maturation due to the presence of the cannabinoids, 20 oocytes were removed from the maturation medium at 0, 5 and 30 min, to evaluate the initial response, and 24 h, to evaluate if the initial response was remaining in order to produce an effect in the mature oocyte, following the same protocol described previously. The primary rabbit polyclonal antibodies against phosphorylated AKT and phosphorylated ERK1/2 (Cell Signaling Technology) were used at 1:100 dilution, and the secondary antibody goat polyclonal Alexa Fluor 488 (Molecular Probes) was used at 1:250. Fluorescence of phosphorylated AKT and phosphorylated ERK1/2 were analysed by confocal microscopy (Leyca TCS-SPE) and quantified using the ImageJ programme v. 1.45 (http://rsb.info.nih.gov/ij) (Huang et al. 2012). Fluorescence was evaluated separately in granulosa cells and oocytes. All experiments were performed in the same day, with the same method and using the same settings in the microscope to be able to compare the fluorescence among treatments. Data are expressed as a fold over vehicle phosphorylation.

**RNA extraction, reverse transcription PCR and real-time PCR (qPCR)**

Gene expression was examined in oocytes and blastocysts as described previously (Bermejo-Alvarez et al. 2011). Pools of 20 oocytes were obtained for the maturation stages as performed for immunofluorescence: GV, MI and MII. For blastocysts, we used pools of 10 obtained on day 7 post-fertilization; three replicates of four samples per experimental group were carried out in both cases. Poly(A) RNA was extracted using the Dynabeads mRNA DIRECT Micro Kit (Ambion, Thermo Fisher Scientific Inc) following the manufacturer’s instructions with minor modifications (Bermejo-Alvarez et al. 2010). After 10 min of incubation in lysis buffer with Dynabeads, poly(A) RNA attached to the Dynabeads was extracted with a magnet and washed twice in washing buffer A and washing buffer B. RNA was eluted with Tris-HCl. After extraction, RT reaction was carried out following the manufacturer’s instructions (Epicentre Technologies Corp, Madison, WI, USA) using poly(T) primers, random primers and MMLV High Performance Reverse Transcriptase enzyme in a total volume of 40 µL, to prime the RT reaction and produce cDNA. Tubes were heated to 70°C for 5 min to denature the secondary RNA structure and then the RT mix was completed with the addition of 50 units of reverse transcriptase. Next, the tubes were incubated at 25°C for 10 min to promote the annealing of random primers, at 37°C for 60 min to allow the RT of RNA and, finally, at 85°C for 5 min to denature the enzyme. Four cDNA samples were performed per experimental group. All qPCR analyses were run in the following conditions: 95°C for 3 min, 35 cycles at 94°C for 15 s, 54°C for 25 s and 72°C for 20 min, followed by a final extension step at 72°C for 5 min. qPCR analyses were carried out in duplicate in the Rotor Gene 6000 real-time cycler (Corbett Research, Sydney, Australia) using 5 ng of each sample to the PCR mix (GoTaq qPCR Master Mix, Promega) containing the specific primers selected to amplify the selected genes. The comparative cycle threshold (CT) method was used to quantify expression levels. Values were normalized to the endogenous control (housekeeping gene: histone H2a1z). According to the comparative CT method, the ΔCT value was determined by subtracting the HK mean CT value for each sample from each gene CT value of the sample. The calculation of ΔΔCT involved using the highest treatment ΔCT value, that is, the treatment with the lowest target expression, as an arbitrary constant to subtract from all other ΔCT sample values. Fold changes in the relative gene expression of the target were determined using the formula 2−ΔΔCT (Murray et al. 2007). The primers used for qPCR are as follows: H2A histone family, member Z (H2AFZ), cannabinoid receptor type 1 (CNR1), cannabinoid receptor type 2 (CNR2), BCL2-associated X protein (BAK), BCL2-like 1 (BCL2), tumour protein p53 (TP53), glutathione peroxidase 1 (GPX1), superoxide dismutase 2, mitochondrial (SOD2), DNA cytosine-5-methyltransferase 3 alpha (DNMT3A), insulin-like growth factor 2 receptor (IGF2R), solute carrier family 2 (facilitated glucose 1 transporter), member 5 (SLC2A5), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), POU class 5 homeobox 1 (Oct3/4) (POUSF1), intereron tau (IFNz) and gap junction protein, alpha 1 (former CX43) (GJA1). Primer sequences and approximate sizes of the amplified fragments of all transcripts are provided in Table 1. The presence or absence of mRNA for CB1 and CB2 was established in maturation stages; H2AFZ was used as the housekeeping gene. Foetal bovine organs like brain and spleen, obtained from the slaughterhouse were used as positive control for expression of CNR1 and CNR2 respectively. RT-PCR with total RNA without reverse transcriptase was used as negative control.

**Data analysis**

All statistical tests were performed using the software package SigmaStat 3.5 (Jandel Scientific, San Rafael, CA, USA). Fluorescence intensity of phosphorylated AKT and phosphorylated ERK1/2, cleavage rates, blastocyst yields and relative mRNA abundances for candidate genes were compared by one-way ANOVA (P<0.05) followed by multiple pair-wise comparisons using the Tukey method.
Results

Expression dynamics and immunolocalization of the cannabinoid receptors CB1 and CB2 during bovine oocyte maturation

During the in vitro maturation of bovine oocytes, CB1 mRNA was detected in GV and MI oocytes but not MII stage oocytes. In contrast, the CB2 transcripts appeared in oocytes in all stages following the resumption of meiosis (GV, MI and MII) (Fig. 1).

Immunofluorescence analysis revealed the presence of both CB1 and CB2 receptor proteins during IVM (Fig. 2). CB1 was evenly distributed in GV stage oocytes yet adopted a peripheral location after the resumption of meiosis in the MI stage and a more evident peripheral location in MII. In contrast, staining for the CB2 receptor was homogeneous across the cell in all the stages examined.

Exposure to HU-210 or THC during bovine oocyte maturation modifies subsequent AKT and ERK1/2 phosphorylation patterns

No differences were found among control groups, with or without 0.1% DMSO, in phosphorylation pattern of

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Figure 1 CB1 and CB2 receptor expression during the nuclear maturation of bovine oocytes. CB1 (305 bp), CB2 (172 bp) and H2AFZ (209 bp) mRNAs in stage GV, MI and MII oocytes matured in vitro. Foetus of bovine species (Fet) served as positive control for CB1 and CB2, brain and spleen respectively. To validate the RT-PCR procedure, reactions with total RNA and without reverse transcriptase were used as negative control (NC). Molecular weights (MW; bp) are indicated on the left. n = 3; a representative RT-PCR experiment is shown.
AKT and ERK1/2 proteins, embryo development (day 9: 24.6 ± 2.6% vs 27.3 ± 4.7% respectively) and blastocysts gene expression. Based on this and on the fact that DMSO was the vehicle for both drugs, it was considered the most appropriate group to be used as a control.

In Fig. 3 it may be observed that no phosphorylation signal was produced for any of the proteins examined before the addition of the drugs (0'). In contrast, while staining for phosphorylated AKT was negligible in oocytes exposed to vehicle (DMSO), after 5 min of treatment with either HU-210 or THC, a clear AKT phosphorylation signal was detected in granulosa cells along with weaker staining in the oocyte. After 30 min, this staining pattern remained fairly stable. However, after 24 h of incubation (the time required for bovine oocyte maturation), pAKT was homogenously distributed across the oocyte and its signal in the granulosa cells diminished in both the HU-210 and THC groups while the absence of pAKT staining persisted in the vehicle group. The pAKT phosphorylation pattern at 24 h was, nevertheless, weaker in the HU-210 than THC group (Fig. 3A).

ERK1/2 phosphorylation patterns (Fig. 3B) in response to 5 min of treatment with the cannabinoid agonists included a pERK1/2 signal in granulosa cells, which was weaker in the vehicle group, and some signal across the oocyte in both the HU-210 and THC groups. However, after 30 min of incubation, pERK1/2 staining in the oocyte significantly differed between the vehicle and treatment groups whereas after 24 h, ERK1/2 phosphorylation similarly diminished in granulosa cells and increased over the oocyte in all the study groups (Fig. 3B).

Changes in velocity of meiotic progression of bovine oocyte’s exposure to HU-210 or THC

To analyse if the exocannabinoids have some effect on oocyte nuclear maturation we fixed oocyte after 0, 2, 4, 6, 8 and 12 h of IVM with media supplemented with HU-210 or THC. Oocytes nuclear stage was classified in GV, GVBD, PMI, MI and MII. As we can see in Fig. 4A, at 0 h all the oocytes were in GV stage, and after 2 h of IVM a higher percentage of GVDB was found when we used exogenous cannabinoids compared with the control group. After 6 h of IVM the majority of the oocytes were at PMI, but no differences were observed among treatments. Also after 8 h of IVM, all groups reached MI (Fig. 4C) at the same time, however, after 12 h of maturation a higher percentage of MII stage was found in both experimental groups compared with control (Fig. 4D).

Blastocyst rate and gene expression of embryo produced from oocytes matured in the presence of HU-210 or THC

We next examined whether exposure to the cannabinoids during oocyte maturation would have impacts on fertilization and/or subsequent embryo development and quality. To first establish the effects of the cannabinoids during oocyte maturation, immature COCs were cultured in vitro for 24 h in the presence or absence of HU-210 or THC. The cannabinoids were then removed from the culture medium and IVF experiments performed on these matured COCs. Incubation with 100 nM of HU-210 or THC had no significant effects on the rates
recorded of cleaved embryos or blastocysts on days 7, 8 and 9 post-IVF ($P > 0.05$, Table 2). To determine effects on embryo quality, we examined the expression of mRNAs for key genes related to apoptosis, oxidative stress, methylation, metabolism, cell junctions, pluripotency and implantation. Expression patterns of the majority of these genes did not differ in embryos arising from oocytes maturated in the presence of THC or HU-210 compared with control ($P < 0.05$, Fig. 5). However, we observed the upregulation in the treatment vs control groups ($P < 0.05$) of the genes GJA1, also known as connexin 43, involved in cell signalling related to the formation of cell junctions, and IFN$\tau$, involved in cell signalling related to recognition for implantation. The expression of IFN$\tau$ was similarly upregulated in the HU-210 and THC groups while, although GJA1 expression was also upregulated in both treatment groups ($P < 0.01$, Fig. 5), this upregulation was significantly more pronounced in the THC group compared with the HU-210 group ($P < 0.01$, Fig. 5).

**Discussion**

The findings of our study indicate that, when present during the *in vitro* maturation of bovine oocytes, the exocannabinoids HU-210 and THC are able to modulate AKT and ERK1/2 phosphorylation. In addition to this effect we observed that the expression of two genes related to embryo quality (IFN$\tau$ and GJA1) was upregulated.

Exocannabinoids such as HU-210 and THC are pharmacologically more stable than endocannabinoids (such as AEA) and are thus the preferred choice for pharmacology assays (Pertwee 2006). Our selection of the bovine oocyte model was based on the fact that a high percentage of oocytes mature *in vitro* compared with other model species (Sirard et al. 2006, Moussa et al. 2015), and their similarities with human oocytes in terms of metabolic requirements, genome activation, interactions with the culture medium and early embryo development (Menezo et al. 2000, Van Hoeck et al. 2011).
Maternal RNA is critical for an efficient meiotic cell cycle and ensuing fertilization and embryo development. Despite this, oocytes suffer a maternal RNA loss of around 20% during their maturation (Chen et al. 2013, Mehlmann 2013). This study detected the dramatic degradation of CB1 mRNA in MII oocytes, as observed in the mouse model (López-Cardona et al. 2014). This event could play a critical role in acquiring oocyte competence for fertilization and subsequent embryo development. At the protein level, we noted changes only in the localization of the CB1 receptor, which moved towards the periphery of the oocyte as meiosis progressed, whereas the localization of the CB2 receptor remains unchanged. Given that receptors need to reach the cell surface in order to act (Cahill et al. 2007) (according to G-protein-coupled receptor theory), CB1 could be the active receptor during oocyte maturation, as reported for the mouse and human models (Peralta et al. 2011, López-Cardona et al. 2014), suggesting that the endocannabinoid system, acting via CB1, is a conserved mechanism in mammalian oocyte maturation.

Oocyte maturation involves the activation of various signal transduction pathways, including PI3K/AKT or MAPK pathways (Schmitt & Nebreda 2002, Cecconi et al. 2012, Conti et al. 2012), which culminate in the activation of maturation-promoting factor (MPF), a complex consisting of cdc2 kinase and cyclin B (Schmitt & Nebreda 2002). Myelin transcription factor 1 (MYT1), the major inhibitory kinase of the MPF complex, is phosphorylated and inhibited by AKT activity, thus promoting the resumption of meiosis (Okumura et al. 2002). In effect, studies in mouse and rat oocytes have shown that AKT activity blockage leads to a significant reduction in cdc2 activity and delayed meiosis resumption (Kalous et al. 2009) and that LH treatment induces higher phosphorylation levels of AKT (Carvalho et al. 2003). Cow oocytes are dependent on active AKT for successful MI/MII transition (Tomek & Smiljakovic 2005). Our results indicate that exposure of these oocytes to HU-210 or THC during their in vitro maturation enhances AKT phosphorylation both in granulosa cells and within the oocyte.

The capacity of FSH or EGF to activate ERK1/2 is well documented (Gonzalez-Robayna et al. 2000, Wayne et al. 2007). In cow, such phosphorylated ERK1/2 is necessary for meiosis resumption (Levesque & Sirard 1995, Su et al. 2003). Here, we show that cannabinoids

<table>
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<th>Table 2</th>
<th>Cleavage rates (D2) and blastocyst yields at day 7 (D9), 8 (D8) and 9 (D9) with and without cannabinoids supplementation in IVM.</th>
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Values are the mean ± s.e.m., 100 mM of HU210 or THC were used, P > 0.05.
have a similar effect to EGF or other promoters of maturation (Conti et al. 2012) on the activation of ERK1/2 both in granulosa cells and in the oocyte. In those oocytes exposed to cannabinoids, meiosis resumption began earlier than in oocytes from the control group. This suggests a potential role for MAPK in the early stages of meiosis resumption in the bovine oocyte model through considerable acceleration of GVBD (Fissore et al. 1996, Kalous et al. 1993). On the contrary, in the mouse oocyte model, cannabinoids do not seem to affect the ERK1/2 phosphorylation status in early maturation stages in which MAPK is not involved (Verlhac et al. 1994, López-Cardona et al. 2014). Effectively, we observed here that after 24 h maturation, ERK1/2 reached its maximum activity in MII-arrested bovine oocytes, whereas in the mouse model, cannabinoids were found to reduce the phosphorylation of ERK1/2 (López-Cardona et al. 2014). Taken together, these results indicate differences among mammalian species in the dynamics of the MAPK pathway, as reported by others (Fissore et al. 1996).

In this study, the embryo development rate to the blastocyst stage was not affected by exposure to HU-210 or THC during IVM, although this treatment did have a beneficial effect on the expression of two genes related to embryo quality, IFNτ and GJA1. IFNτ is responsible for the establishment and maintenance of early pregnancy in cattle. Accordingly, bovine blastocysts produced in vivo, in vitro or through ICSI show the differential expression of IFNτ (Gutierrez-Adan et al. 2004, Arias et al. 2015) with in vitro-produced blastocysts featuring lower relative IFNτ transcription compared with their in vivo counterparts (Lonergan et al. 2003). Connexin43 (GJA1) is associated with the formation of cell junctions and several reports suggest that its expression is lower in blastocysts produced in vitro vs in vivo (Rizos et al. 2002, Gutierrez-Adan et al. 2004, Tesfaye et al. 2007). Hence, the significantly upregulated expression of both IFNτ and GJA1 observed here for embryos supplemented with cannabinoids during oocyte maturation suggests that cannabinoids may have improved the quality of those embryos.

In conclusion, our findings indicate that exposure to cannabinoids during the in vitro maturation of oocytes activated AKT and ERK1/2 faster and it is possible that activation could serve to modulate PI3K/AKT and MAPK molecular pathways, probably via CB1 activation, with positive effects on the expression of genes related to embryo quality and no repercussions on the embryo development. These findings have implications for improving IVM in cattle. In addition, the bovine oocyte model emerged as a useful tool for preclinical screening of exogenous cannabinoids targeted at improving the quality of human embryos produced through assisted reproduction techniques.

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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