Failure to launch: aberrant cumulus gene expression during oocyte in vitro maturation

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Abstract

In vitro maturation (IVM) offers significant benefits for human infertility treatment and animal breeding, but this potential is yet to be fully realised due to reduced oocyte developmental competence in comparison with in vivo matured oocytes. Cumulus cells occupy an essential position in determining oocyte developmental competence. Here we have examined the areas of deficient gene expression, as determined within microarrays primarily from cumulus cells of mouse COCs, but also other species, between in vivo matured and in vitro matured oocytes. By retrospectively analysing the literature, directed by focussing on downregulated genes, we provide an insight as to why the in vitro cumulus cells fail to support full oocyte potential and dissect molecular pathways that have important roles in oocyte competence. We conclude that the roles of epidermal growth factor signalling, the expanded extracellular matrix, cumulus cell metabolism and the immune system are critical deficiencies in cumulus cells of IVM COCs.


Introduction

In vitro maturation (IVM) of oocytes is a potentially valuable technology for both clinical infertility treatment and assisted reproductive technologies in animals (Gilchrist & Thompson 2007). IVM has been and remains a valuable research tool in understanding factors that irrefutably influence oocyte developmental competence. Indeed, the great majority of studies investigating the regulation of oocyte competence are performed in vitro. Through such studies, it is clear that the cumulus has substantial influence on oocyte biology, including (but certainly not exclusively): the supply of small molecular weight molecules involved in the regulation of meiotic maturation and metabolism of the oocyte; the acquisition of cytoplasmic signalling mechanisms enabling further development by the oocyte; the induction of sperm capacitation enabling penetration of the oocyte. This is clearly observed when developmental competence is compared between cumulus-enclosed and denuded oocytes during IVM (e.g. Younis & Brackett 1991, Hazeleger et al. 1995, Zhang et al. 1995, Luciano et al. 2005), where denudation results in a significant drop in developmental capability. Furthermore, this is not attributed to simply poor fertilisation, as parthenogenic activation rates differ in oocytes denuded of cumulus cells prior to maturation compared with intact COCs in the presence of FSH (Chian et al. 1995).

Despite several decades of research, IVM-derived oocytes of most species have a perceptively poorer developmental outcome than those derived following in vivo maturation, even despite recent advances suggesting the gap is closing (Walls et al. 2012, 2015). Bi-directional communication between the cumulus vestment and oocyte is critical for oocyte competence and has been the subject of many previous reviews (Gilula et al. 1978, Gilchrist et al. 2008, Kidder & Vanderhyden 2010). However, this communication is under stress during in vitro maturation relative to in vivo maturation. We proposed that, compared to in vivo derived COCs, the cumulus cells of an in vitro matured COC would exhibit significantly different gene expression patterns which may indicate aspects of cumulus cell function that are deficient, therefore leading to reduced oocyte quality. Our analysis was primarily informed by the differential expression between in vivo and in vitro mouse cumulus cell genes published not only by Kind et al. (2013), but also by Tesfaye et al. (2009) (bovine CC) and Ouandaogo et al. (2012) (human CC). We acknowledge that variations in species and in methodology used for the generation
of both in vivo and in vitro matured COCs may cause differences in the cumulus cell transcriptome, and that differences in microarray platforms will contribute a significant source of variation in results. Our intent is not to discuss closely the results of these microarrays, but use them as a guide to direct us to the major networks in cumulus cells leading to reduced developmental competence of oocytes in vitro (Fig. 1). In doing so, we have focussed on major distinct physiological processes with evident dysregulation because of IVM, and expand to what is known about their role in oocyte maturation.

### Epidermal growth factor receptor signalling

Of the seven recognised cognate epidermal growth factor-like ligands (EGF-L), three are known to be expressed in granulosa cells and COCs; amphiregulin (AREG), epiiregulin (EREG) and betacellulin (BTC), and all were significantly reduced in IVM COCs compared to in vivo (Kind et al. 2013). Deficient expression of EGF-L factors in IVM has been confirmed in a number of subsequent studies (Richani et al. 2013, 2014b). Additionally, Versican is another factor with demonstrated EGF-like activity in COC (Dunning et al. 2015), which was also reduced in IVM compared to in vivo COCs as has been previously demonstrated (Dunning et al. 2007). The EGF signalling pathway is fundamental for oocyte maturation and this has been established over several decades (Downs et al. 1988). More recently, the specific importance of AREG, EREG and BTC was demonstrated (Park et al. 2004). The EGF-L factors and Versican are rapidly (within 1 h) induced in granulosa cells responding to the LH-surge and transmit the ovoidal signal to cumulus cells which are not directly LH responsive (Eppig et al. 1997). This important intermediary paracrine role played by granulosa cell derived EGF-L factors in activation of oocyte maturation has been confirmed using whole follicle culture (Ashkenazi et al. 2005) and genetic dissection (Hsieh et al. 2011). Likewise, a role for granulosa-derived Versican in expansion of the COC matrix has been demonstrated (Dunning et al. 2012, 2015). However it is also clear that once maturation of the COC is activated by granulosa derived factors, these same factors are subsequently induced in cumulus cells and hence likely to maintain an autocrine activation of the EGF signalling pathway throughout oocyte maturation, ovulation and fertilisation (Shimada et al. 2006b). Expression of these factors in cumulus cells has been shown to associate with oocyte developmental potential (Gebhardt et al. 2011, Huang et al. 2015), and their deficient expression in IVM systems is therefore likely to be a key contributor to lower quality outcomes. Recently it has come to light that the EGF receptor activation in cumulus cells stimulates PI3K/AKT signalling in the oocyte which controls protein translation and developmental competence (Chen et al. 2013, Franciosi et al. 2016). The increase in protein translation promotes release of factors such as IL7 from oocytes which act back on cumulus cells (Cakmak et al. 2016). Exactly how the cumulus cell response is transferred to the oocyte remains unknown, but the importance of EGF-L induction in granulosa cells and hence the importance of replicating this pathway in vitro is clear.

These findings have initiated a trend towards including EGF or EGF-L factors in stimulated IVM systems. A number of studies have undertaken comparison of IVM outcomes using treatment with FSH vs EGF or each of the EGF-L factors as COC maturation stimuli. While more evaluation of dose responses are required to properly compare the activity of each factor in IVM COCs, the consensus findings indicated that when compared at 50 ng/mL, recombinant EREG had the greatest beneficial effects on mouse COC glycolysis (Sugimura et al. 2014), oocyte mitochondrial activity (Richani et al. 2014a) and embryo developmental potential (Richani et al. 2014b), although why EREG was better at promoting these processes is undetermined. Other studies have suggested AREG may play the most important role in oocyte maturation. A non-redundant
contribution of AREG to mouse oocyte maturation in vivo was illustrated by null mutation of AREG causing delayed meiotic progression (Hsieh et al. 2007) and a significant 40–50% reduction in the proportion of oocytes that could achieve fertilisation (Chen et al. 2013). Likewise in human and rhesus monkeys AREG protein was found to be more abundant (median 30 ng/mL, 36 h after hCG) than other EGF-L factors in the follicular fluid of mature follicles (Zamah et al. 2010, Peluffo et al. 2012). The concentration of AREG in follicular fluid was correlated with oocyte developmental potential (Zamah et al. 2010), however, potential juxtacrine signalling within COC by AREG or the other EGF-L is not considered in this approach investigating the free ligand in antral fluid which may indeed be less biologically relevant. A dose–response analysis in porcine COCs showed that AREG at 100 ng/mL significantly increased oocyte maturation to MII (Sugimura et al. 2015). Another recent study found AREG mRNA induction in human granulosa cells or in COC correlated with ICSI success parameters including numbers of oocytes retrieved, meiotic progression, fertilisation and embryo quality (Huang et al. 2015). These studies together suggest that AREG may be most important and should be tested in primate IVM.

The normal in vivo induction of EGF-L factors involves sequential increases in the three factors with overlapping but different kinetics. This may promote a specific temporal pattern of signalling in the COC which is difficult to replicate in IVM and the physiological importance of this sequence for full oocyte developmental potential is unknown. Supplementation of IVM cultures with EGF promotes EGF-L mRNA expression to levels quantitatively similar to in vivo COCs, yet levels of EGF receptor phosphorylation at Tyrosine Tyr1068 remain generally lower than in vivo and differ in temporal kinetics (Richani et al. 2013). Variable responses to the different EGF-L ligands are mediated in part through interaction with distinct receptors or receptor complexes. All seven cognate EGF related factors can bind and activate EGF-receptor (EGFR or ERBB1), but some also bind other members of the ERBB receptor family, such as BTC which can activate all the ERBB members (Dahlhoff et al. 2014). Likewise Neuuregulin 1 (NRG1) is an EGF-like factor induced in granulosa cells during oocyte maturation in vivo (Noma et al. 2011), but which binds only ERBB2 and ERBB3, not ERBB1. The rapid induction of Nrg1 in granulosa cells may initially suppress oocyte maturation and has thus been proposed to be an important granulosa-derived factor helping to synchronise maturational events in vivo (Kawashima et al. 2014). Because Nrg1 is not a cumulus gene product it is not found among differentially expressed genes in IVM COC; however, it is deficient due to the absence of granulosa cells and may explain the abnormally rapid meiotic maturation of IVM oocytes (Kawashima et al. 2014).

In addition to the different ligand-receptor affinities, variable phosphorylation of intracellular Tyr residues on the EGF receptors is activated by the different ligands (Wilson et al. 2012), with important distinctions in internalisation and recycling of the receptors and affecting the signalling duration. Amphiregulin promotes receptor recycling to epithelial cell membranes, while EGF and BTC traffic the receptor to lysosomes for degradation (Roepstorff et al. 2009), which will truncate signal duration and blunt the cell’s capacity to respond to other EGF-L whether exogenously added or endogenously produced. This outcome is consistent with what has been described for EGFR Tyr1068 phosphorylation in IVM COC, which show a sharp rapid induction, but premature downregulation (Richani et al. 2013). Finally, some of the EGF-L ligand's signalling is modulated by heparan sulphate proteoglycans on the cell surface or the local ECM environment. Amphiregulin action is dependent on heparan sulphate (Mahtouk et al. 2006), and the expression of many HSPG is altered under IVM conditions (Watson et al. 2012). Together all these differences weave a tapestry of complex signal transduction consequences contingent on the mix and timing of ligands and receptors present. The specific combination and timing of EGFR signalling in COC maturation is clearly important, but has yet to be investigated in detail. Likewise, the involvement of HSPG in cumulus cell signalling is just emerging (Watson et al. 2012). The collective deficiency in expression of AREG, EREG and BTC and other signalling molecules is clearly suboptimal and likely to result in lower autocrine activation of the EGF pathway in IVM COCs.

An alternative IVM technology that uses a pre-maturation treatment to elevate cAMP before stimulating COC with FSH and/or EGF, improved oocyte competence in cows (Albuz et al. 2010) and mice (Zeng et al. 2014) and enhances EGF-L peptide expression (Richani et al. 2013, 2014b). However, ERK1/2 phosphorylation, which is a downstream consequence of EGF-L activity, was not different (Richani et al. 2013, 2014b). The results are suggestive that increased EGF-L production caused by elevated cAMP activated signalling in the prematuration phase was responsible for higher oocyte developmental competence (Richani et al. 2014b).

Versican is profoundly deficient in IVM COC in mouse (Dunning et al. 2007) and its expression in human cumulus cells has been repeatedly found to be associated with successful ART outcomes (Gebhardt et al. 2011, Wathlet et al. 2011, Ekart et al. 2013, Hammond et al. 2015). The capacity of Versican to activate EGFR in cumulus cells and promote COC maturation was also recently discovered (Dunning et al. 2015). While the ability of Versican to bind and activate EGFR is known (Du et al. 2010), its specific downstream signal transduction mechanism is unknown. Importantly, the temporal kinetics of gene
induction mediated by Versican in COC was more similar to the in vivo pattern than that achieved by EGF treatment (Dunning et al. 2015). Of particular interest, induction of Ere2 and Ptgs2 by Versican was more sustained than with EGF (Dunning et al. 2015) mimicking more closely the expression profile observed in vivo (Shimada et al. 2006b). Based on the sustained signalling capacity of Versican, we believe it to be an important component of the periovulatory signalling cascade and that its absence during IVM impacts on oocyte quality. It will be of interest to determine whether Versican has similar signalling capacity in human cumulus cells and whether this is a key missing aspect of EGF pathway activation important for high oocyte developmental potential after IVM.

Together these findings on the role of EGF-L factors in activation of COC maturation and the evident importance of local EGF-L production in COC for acquisition of full oocyte developmental potential suggest that the lower EGF-L production in IVM is expected to be one aspect that has a negative influence on developmental potential. The most plausible explanation at present is that a vicious cycle arises whereby key mural granulosa cell derived contributors to COC maturation are absent in vitro resulting in deficient expression of critical factors (e.g. EGF-L, Versican, or HSPG), which further diminish the activation of key signalling pathways in the IVM COC.

Extracellular matrix

The in vivo action of the ovulatory LH-surge also stimulates production of an extensive extracellular matrix in the COC. Again this requires granulosa cells which produce a variety of factors that impinge on cumulus cells resulting in the induction of ECM gene expression while some granulosa cell secreted proteins (e.g. versican and Adamts1) are directly incorporated into the COC matrix (Russell & Salustri 2006, Russell & Robker 2007). The resultant phenomenon, known as cumulus expansion, occurs concomitantly with oocyte meiotic maturation and is required for ovulation (Russell & Robker 2007). Cumulus expansion has been described in nearly all mammalian species investigated to date, yet historically its role was thought to have been restricted to its functionality during oocyte pick-up by the infundibulum (Odor & Blandau 1973, Lam et al. 2000) and during fertilisation in the oviduct (Salustri et al. 2004). In recent years studies have shown that formation of a compositionally complete and functional cumulus matrix is associated with oocyte quality (McKenzie et al. 2004, Cillo et al. 2007, Yung et al. 2010, Gebhardt et al. 2011, Wathlet et al. 2011, Ekart et al. 2013), oovulation rate (Varani et al. 2002, Kulbert et al. 2003, Ochners et al. 2003, Mittaz et al. 2004, Brown et al. 2006), cumulus cell signalling (Dunning et al. 2015), adhesive and invasive capacity of the COC (Akison et al. 2012) and molecular filtration (Dunning et al. 2012). Importantly, the cumulus matrix formed during in vitro maturation is compositionally and functionally altered and may account for the poorer quality of oocytes produced using this technology (Dunning et al. 2007, 2012, Kind et al. 2013).

In vivo, the EGF-L peptides secreted by granulosa cells, as described above, act on cumulus cells to induce genes such as prostaglandin endoperoxide synthase 2 (Ptgs2) involved in cumulus matrix gene expression and hyaluronan synthase (Has2), tumour necrosis alpha induced protein 6 (Tnfaip6), pentraxin 3 (Ptx3). These matrix components interact, along with versican from the granulosa cells and inter-alpha trypsin inhibitor heavy chain from circulation, to assemble the COC matrix (Russell & Salustri 2006). Another granulosa product, Adamts1 is progesterone-dependently induced in granulosa cells following the LH-surge and found in abundance in the expanded COC (Russell et al. 2003a,b). In vitro it is clear that expression of cumulus matrix genes is truncated or temporarily altered (Jones et al. 2008, Wells & Patrizio 2008, Tesfaye et al. 2009, Kind et al. 2013, Dunning et al. 2015) and this is associated with the poor oocyte developmental competence. Cumulus expansion also requires calpain-mediated migration of cumulus cells along the newly synthesised cumulus matrix (Kawashima et al. 2012). Whether cumulus cell motility during IVM is altered and functionally impacts oocyte quality is yet to be determined.

We have shown that following IVM the expanded mouse COC is completely void of the cumulus matrix proteins ADAMTS1 and Versican despite their abundance in the in vivo matured COC (Dunning et al. 2007). The absence of these proteins and the reduced abundance of other cumulus matrix components results in a compositionally deficient and functionally altered matrix. We have shown that the in vivo matured matrix is able to restrict diffusion of metabolites to the oocyte such as glucose and sequester locally produced signalling molecules including prostaglandin E2 (Dunning et al. 2012). This molecular filtration property of the cumulus matrix is however deficient following IVM (Dunning et al. 2012). The ability of the cumulus matrix to regulate metabolite supply while retaining cumulus cell synthesised signalling factors is likely to affect oocyte quality. It is logical that the loss of the ECM capacity to retain and concentrate locally produced factors contributes to poor IVM quality since the gene products involved in prostaglandin synthesis, oocyte secreted growth factors and glucose metabolism are associated with oocyte quality, and supplementation of IVM media with the products of these pathways improves oocyte quality (Hussein et al. 2005). Thus, the reduced oocyte quality following IVM may be due to dysregulated control of oocyte exposure to metabolites and signalling molecules. Whether similar deficiencies in cumulus matrix filtration occurs following IVM of human COCs.


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has not been determined, nor whether repletion of the mouse cumulus matrix through exogenous addition of proteins such as Versican restores this function.

Extracellular matrix proteins can also function as signalling molecules. As described above, recombinant Versican induces cumulus-specific gene expression during IVM (Dunning et al. 2015). Other ECM proteins such as heparan sulphate proteoglycans can also either directly transduce an intracellular signal (e.g. Syndecans) or modulate signalling by EGF, FGF or TGFβ family ligands.

That the cumulus matrix is important for oocyte quality is clearly supported by several studies associating human cumulus ECM gene expression with oocyte developmental potential. The expression of Versican (VCAN) has been positively correlated with oocyte developmental potential in four independent studies including a positive association with live birth (Gebhardt et al. 2011, Wathlet et al. 2011, Ekart et al. 2013, Hammond et al. 2015). Similar positive associations have been shown for Ptgs2, Adamts1 and Has2 (McKenzie et al. 2004, Cillo et al. 2007, Yung et al. 2010, Xu et al. 2015). These retrospective results have yet to be confirmed using randomised controlled trials; however, the independent findings at different sites of consistent associations between cumulus matrix gene expression and oocyte developmental outcomes provide strong evidence that formation of an appropriate cumulus matrix is essential for human oocyte quality. Whether human cumulus expression of matrix genes is similarly reduced during IVF as occurring in the rodent is yet to be determined and should be investigated.

Metabolism

The importance of the cumulus cell supply of nutrients and substrates to the oocyte to achieve developmental competence has been increasingly acknowledged over the last two decades (Dumesic et al. 2015). Metabolism of cumulus cells is an important contributor to the bi-directional communication between the oocyte and cumulus vestment (Eppig 1991, Albertini et al. 2001, Matzuk et al. 2002). The environment to which a COC is exposed to during maturation, both in vivo and in vitro, largely impacts its metabolism and developmental competence (Krisher 2013, Dumesic et al. 2015). For example, maternal hyperglycaemia and hyperlipidaemia compromise COC health, embryo development and pregnancy outcomes (Chang et al. 2005, Leroy et al. 2008, Robker 2008, Purcell & Moley 2011, Van Hoeck et al. 2011). It is well established that the oocyte predominantly utilises oxidative phosphorylation, whereas the cumulus vestment has a high rate of glycolytic activity (Thompson et al. 2007). The primary substrate of cumulus cells is glucose and is metabolised via numerous pathways to provide energy, substrates for ECM, nucleic acid synthesis and stress/fuel sensing (reviewed by Sutton-McDowall et al. 2010). Therefore the discovery that several genes in the glucose metabolic pathway have reduced expression during IVM suggests glycolytic deficiency is likely to negatively impact on oocyte developmental potential. These included Slc2a1 (facilitated glucose transporter 1), lactate dehydrogenase (Ldhcl), enolase (Eno2) and hexokinase (Hk2) expression and indicate a reduced glucose uptake and metabolism (Kind et al. 2013). Cumulus cell glucose metabolism is regulated by oocyte-secreted factors, which influence the expression of such genes (Sugiura & Eppig 2005, Sugiura et al. 2005, 2007), and the array results are in line with a reduction in OSF expression during IVM (Mester et al. 2015). Furthermore, glucose (and glutamine)-dependent hyaluronic acid matrix production is most likely inhibited by reductions in glutamine-fructose-6-phosphate transaminase (Gpt1) and hyaluronic acid synthase 2 (Has2) expression within in vitro matured cumulus cells (Caixeta et al. 2013). This is despite many IVF systems utilising follicle stimulating hormone, a potent stimulator of cumulus glucose metabolism and nuclear maturation within COCs (Downs et al. 1996, Sutton-McDowall et al. 2004).

Fatty acid metabolism via beta-oxidation is a potent source of cellular energy. The importance of beta-oxidation during COC maturation is becoming increasingly recognised. In vivo, the LH-surge results in a significant increase in beta-oxidation-related genes in COCs and functional assays demonstrate a significant increase in beta-oxidation during in vitro maturation (Dunning et al. 2010). Further, pharmacological inhibition of beta-oxidation impairs oocyte developmental competence, while promotion with l-carnitine during IVF improves subsequent embryo development in several species (Dunning et al. 2010, 2014, Dunning & Robker 2012). Thus it appears that supply of a fatty acid milieu and necessary co-factors such as l-carnitine that replicates the in vivo environment is likely to result in improved oocyte maturation conditions in vitro.

An emerging interest is the negative impact on oocyte competence of 2-carbon glucose metabolic products, such as glyoxal and methylglyoxal (reactive carbonyl species, RCS) (Tatone et al. 2010, 2011). RCS are derived from glycolysis and the polyl pathway, plus other sources, and are damaging in that they promote lipid peroxidation (Tatone et al. 2010, 2011). Reduction in 2-carbon derivative detoxification enzymes, such as carbonyl reductase (NADPH) 3 (Cbr3) within in vitro matured cumulus cells is evidence that 2-carbon toxicity may occur (Tatone et al. 2010, 2011), and this area requires more attention.

We have recently reported that haemoglobin A1 and B expression (Hba-a1 and Hbb, respectively) greatly differed in mouse cumulus cells derived from in vivo or in vitro maturation, where they were at nearly undetectable levels following in vitro maturation (Kind et al. 2013). Indeed, Hba-a1 was the most
differentially expressed gene from the microarray analysis. This was validated by a substantial loss of HBA protein following in vitro maturation (Kind et al. 2013, Brown et al. 2015). Significantly, this difference in gene expression is supported for both HBA1 and HBB within in vivo vs in vitro matured human cumulus cells (Ouandaogo et al. 2012) (Supplementary Table within publication) and for HBB in bovine cumulus cells (Tesfaye et al. 2009). Our analysis of the temporal gene expression pattern following the ovulatory luteinising hormone (LH) surge demonstrated these two genes were hormonally regulated, with the peak expression level occurring 12-h post hCG. Haptoglobin, required for iron-recycling from haem degradation, also followed this pattern of expression (Brown et al. 2015). Other gene products involved in haem synthesis were also detected, but not hormonally regulated.

The function of haemoglobin within the follicular cells of antral follicles has yet to be determined, but as its presence is hormonally regulated and extends to the oocyte itself (Brown et al. 2015), we have proposed it plays a role during the ovulatory period (Thompson et al. 2015), perhaps involving O2 and/or NO binding and sequestering. A further potential role is as an antioxidant (Thompson et al. 2015). Work is continuing to investigate these options by developing purposefully designed molecular tools to measure haemoglobin-gas interactions.

In addition to haemoglobins and haptoglobin, there are several well-characterised O2-sensitive genes that are upregulated within mouse in vivo matured cumulus cells relative to in vitro matured cumulus cells. These include: endothelin 2 (Edn2); enolase 2 (Eno2); BCL2/adenovirus E1B interacting protein 1, NIP3 (Bnip3); solute carrier family 2 member 1 (Slc2a1); lactate dehydrogenase D (Ldhd); hexokinase 2 (Hk2); N-myc downregulated gene 1 (Ndrg1), NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2 (Ndufa4l2) and vascular endothelial growth factor A (Vegfa) (Kind et al. 2013). Such a pattern of expression points to involvement of the hypoxia inducible factor (HIF) family of transcription factors in regulating cumulus cell function, especially in low levels of dissolved O2. HIF transcription factors are now understood to at least partly mediate gonadotrophin signalling in the ovarian follicle, especially granulosa cells signalling following the luteinising hormone surge (reviewed by Thompson et al. 2015). We have experimentally demonstrated that maturation of COCs in vitro under low O2 concentrations (2% and 5%) involves HIF-induced transcription activity within cumulus cells. Similar upregulation of O2-sensitive genes in bovine cumulus cells is observed following maturation under 5% O2 relative to 20% O2 (Bermejo-Alvarez et al. 2010). Does this suggest that in vitro maturation should be performed at low pO2 (specifically 5%)? Several publications support the concept that IVM under low pO2 conditions improves oocyte maturation, including human IVM (Hashimoto et al. 2000a, Iwamoto et al. 2005, Preis et al. 2007, Walls et al. 2015). Nevertheless, long-term follow-up studies are virtually absent, with the study of Banwell et al. (2007) being the exception. This study reported improved embryo development from low O2 IVM, but post-transfer outcomes were impacted dependent on O2 concentration with, surprisingly, 5% O2 IVM derived foetuses with poorer developmental indices relative to 2% or 20%. A feature of this latter study is that several O2 concentrations for IVM were assessed. In contrast, virtually all other studies investigate 5% vs ‘air’ O2 concentrations. The historical origin for 5% O2 gas applied to gamete and embryo culture derives from work performed several decades ago showing it was optimal for mouse embryo culture (Quinn & Harlow 1978). Whether it has relevance or not to IVM in any species, in particular human IVM, has not been properly assessed. Furthermore, if haemoglobin does have a role to sequester gases, does this impact the optimal IVM gas composition when haemoglobin is present during IVM conditions, as suggested by Brown et al. (2015)? An often touted reason for the use of 5% O2 atmosphere during IVM is the reduction in reactive oxygen species (ROS) production, therefore safeguarding the integrity of chromatin conformation and prevention of lipid and protein oxidation within the maturing oocyte from ROS-induced damage (Hashimoto et al. 2000a, b, Salavati et al. 2012). Therefore it would appear that a lower than atmospheric O2 concentration is preferable, but which concentration is optimal will depend on numerous factors, such as species, composition of medium and the incubation environment as a whole.

Reduced glutathione (GSH) is an essential peptide determining oocyte competence (Sutovsky & Schatten 1997, Furnus et al. 1998, de Matos & Furnus 2000, Bing et al. 2002). Additionally glutathione peroxidase-1 (GPX1) expression in cumulus cells was also recently shown to be associated with human oocyte competence (Ceko et al. 2015). Metabolism in the cumulus cells is directly linked to GSH levels in the oocyte (Xie et al. 2016). Increasing intra-oocyte GSH levels by the addition of component amino acids, especially cysteine, into in vitro maturation medium formulations is a common strategy to improve embryo production (de Matos & Furnus 2000). Several studies demonstrate cumulus cells are a source of reduced GSH for the oocyte, especially while gap junctions between oocyte and cumulus cells are patent (Curnow et al. 2008, 2010, Furnus et al. 2008). Glutathione-S-transferase (Gsta) is a key enzyme in the process of reducing oxidised glutathione, thus regenerating GSH levels. It is therefore pertinent that microarrays (e.g. Kind et al. 2013, Salhab et al. 2013) revealed a reduced level of Gsta transcription within the cumulus of in vitro matured COCs.

With the exception of cholesterol metabolism and steroid synthesis, little is known of lipid synthesis and metabolism within cumulus cells. Much of the focus has
been directed towards the oocyte itself (Thompson et al. 2014). Recent insights reveal that compared to lipid within the oocyte, cumulus cell lipid composition is most vulnerable to exogenous factors such as oxidants, suggesting the cumulus lipid content is ‘protecting’ oocyte cytoplasmic and membrane lipids (Aardema et al. 2013, Lolicato et al. 2015). Microarray data from human and mouse comparing cumulus cell transcriptome between in vitro and in vivo maturation highlight differences in some lipid synthesis and metabolism genes (such as Acox1, Acox4, Alox5ap, Lpgat1, and Fabp4as) (Ouandaogo et al. 2012, Kind et al. 2013). However, studies on how this impacts lipid composition have not followed-up and is a deficiency in our understanding.

The immune system

Hypotheses surrounding the similarities between the oocytes and an immune-regulated event have been proposed over several decades (Espey 1980). While latter work suggested an inflammatory-like state was initiated following the LH-surge (Richards et al. 2002), more recent insights also implicated the involvement an innate, immune-like process (Richards et al. 2008). Research has proposed that an endocrine–immune–cytokine network exists within the pre-ovulatory follicle and involving not only inflammatory molecules but also innate immune molecules within the pattern-recognition receptor family.

While several gene expression studies explore the presence and abundance of a number of ‘immune-cell pathway genes’, little functional work has been conducted. Microarray data on cumulus cells during in vivo maturation and expansion of the cumulus oocyte complex identified a number of genes as upregulated over this period (Adam8, Cd28, Pdcd1, Cdc34, Cdk2, Cd81, Cd97, Emr1, Cd97a, Cd147, Cd166 (Alcam), Cxcr4, Il6, Nrp1, Ptx3, Runx1, Saa3) (Hernandez-Gonzalez et al. 2006), with most never described within the ovary. It is significant that our comparison of COCs derived in vivo and in vitro identified a number of ‘immune-cell pathway genes’ that were dramatically lower in the in vitro derived COCs (Kind et al. 2013).

Perhaps the most widely explored immune modulator in the COC is Interleukin-6 (IL6). IL6 is significantly upregulated during the oocytes cascade in vivo (Hernandez-Gonzalez et al. 2006), and known to be regulated during the follicular phase and present in high levels in the periovulatory follicular fluid in humans (Baskind et al. 2014). Liu et al. (2009) explored a functional role for IL6 in the mouse COC during IVF. They reported a short-term induction of Il6 mRNA in culture that was upregulated by AREG, FSH and PGE2 (4h hCG, Liu et al. 2009), although longer-term regulation in vitro was not explored. The induction was blocked both by indomethacin and RU486, suggesting IL6 is a downstream target of PGE2, and that its expression is modulated by progesterone receptor (Pgr). With a number of these key components dysregulated during IVF (Ptgs1, Ptges, Ptger4, Star, Cyp11a1) (Kind et al. 2013), it is perhaps no surprise that there are alterations in IL6 signalling as a consequence of in vitro maturation. Interestingly, addition of IL6 to IVF was able to promote cumulus expansion in mouse COCs, albeit at concentrations much higher than detectable in follicular fluid; 100–1000ng/ml (Liu et al. 2009) vs less than 1–100pg/ml detected in human follicular fluid) (Buscher et al. 1999, Altun et al. 2011, Baskind et al. 2014).

A limited amount of data in human suggest that the COC may contain CD4+ T cells, which produce both IL4 and LIF (Piccinni et al. 2001), while a number of chemo-attractants for immune cells including monocytes, dendritic cells and natural killer cells have also been found (Cxc14, Kind et al. 2013). In addition to this, a number of genes best described as immune cell markers have been found in the COC in vivo, and dysregulated in vitro. Cd83, a marker of fully matured dendritic cells was decreased following in vitro maturation, while Cd14, a co-receptor for toll-like receptors (TLR), which recognise pathogen-associated molecular patterns (PAMPs), as well as endogenous damage associated molecular patterns (DAMPs) was also decreased in vitro, but present and regulated by LH (hCG) in vivo (Shimada et al. 2006a). Indeed fragmental hyaluronan and Versican (Kim et al. 2009, Hu et al. 2015) are strong DAMP signals. We propose that the production of inflammatory cytokines and/or immune like behaviour of cumulus cells could represent a final checkpoint prior to ovulation, or at fertilisation, when tissue damage releases DAMP signals that impinge on TLRs and thus are absent when oocytes undergo IVM. As such, IVF followed by intra-cytoplasmic sperm injection (ICSI), where the COC is not exposed to sperm or seminal plasma, may represent a further insult. Future experiments comparing in vivo fertilisation, IVF and ICSI may provide valuable insight into the role of immune factors during oocyte maturation and at fertilisation.

Conclusion

IVM remains at the fringe of acceptance both clinically and for animal breeding, as it remains less efficient relative to other embryo production strategies. It occupies several niche applications, but is far from widely accepted. Our analysis of cumulus cell transcriptome reveals many different molecular attributes that separate the functionality of cumulus cells from in vivo and in vitro matured COCs, providing a view of the molecular mechanisms behind the wide discrepancy in capacity to generate developmentally competent and mature oocytes. Consistent evidence showing deficient EGF-L signalling, ECM composition and function, metabolism and immune gene expression in IVF as
well as demonstrating the importance of these pathways in oocyte maturation lead us to conclude that attention given to understanding these molecular mechanisms and to restoring them in IVM will lead to improved IVM efficacy. It is likely an unattainable goal to achieve parity in the transcriptome of these two sources of oocytes, although management of cAMP levels, use of EGF-L peptides, inclusion of specific growth factors, such as cytokines, oocyte secreted factors, and other growth factors (including Versican), providing the correct oxygenation and substrate composition in the medium, will contribute to normalising cumulus cell function and oocyte developmental competence.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References


Calmak H, Franciosi F, Zamah AM, Caders MI & Conti M 2016 Dynamic secretion during meiotic reentry integrates the function of the oocyte and cumulus cells. PNAS 113 2424–2429. (doi:10.1073/pnas.1519900113)


de Matos DG & Furnus CC 2000 The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development


www.reproduction-online.org


Russell DL, Doyle KM, Ochsner SA, Sandy JD & Richards JS 2003 Processing and localization of ADAMTS-1 and proteolytic cleavage


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