

The pivotal role of glucose metabolism in determining oocyte developmental competence

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Abstract

The environment that the cumulus oocyte complex (COC) is exposed to during either *in vivo* or *in vitro* maturation (IVM) can have profound effects on the success of fertilisation and subsequent embryo development. Glucose is a pivotal metabolite for the COC and is metabolised by glycolysis, the pentose phosphate pathway (PPP), the hexosamine biosynthesis pathway (HBP) and the polyol pathway. Over the course of oocyte maturation, a large proportion of total glucose is metabolised via the glycolytic pathway to provide substrates such as pyruvate for energy production. Glucose is also the substrate for many cellular functions during oocyte maturation, including regulation of nuclear maturation and redox state via the PPP and for the synthesis of substrates of extracellular matrices (cumulus expansion) and O-linked glycosylation (cell signalling) via the HBP. However, the oocyte is susceptible to glucose concentration-dependent perturbations in nuclear and cytoplasmic maturation, leading to poor embryonic development post-fertilisation. For example, glucose concentrations either too high or too low result in precocious resumption of nuclear maturation. This review will discuss the relevant pathways of glucose metabolism by COCs during *in vivo* maturation and IVM, including the relative contribution of the somatic and gamete compartments of the COC to glucose metabolism. The consequences of exposing COCs to abnormal glucose concentrations will also be examined, either during IVM or by altered maternal environments, such as during hyperglycaemia induced by diabetes and obesity.

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Introduction

The immature oocyte remains in an arrested state from the completion of oogenesis during foetal development, and oocyte maturation is completed within antral stage follicles in post-pubertal mammals in response to the ovulatory surges in gonadotrophins (Fig. 1A). Alternatively, spontaneous completion of maturation will occur following mechanical release of the oocyte from ovarian antral follicles (Edwards 1965). This latter phenomenon is referred to as *in vitro* oocyte maturation (IVM) and is commonly used to study oocyte development in several mammalian species, and has application to assisted reproduction in humans and livestock animal production (Trounson *et al.* 2001, Gilchrist & Thompson 2007, Hashimoto 2009).

Oocyte maturation involves the resumption of meiosis from prophase I (germinal vesicle stage, GV) to the extrusion of the first polar body (metaphase II, MII; Fig. 1B and C); expansion of the surrounding somatic cell compartment (cumulus cells) and maturation of the cytoplasm to support fertilisation and early embryonic development. As mentioned, the oocyte is surrounded by numerous layers of cumulus cells forming the

cumulus oocyte complex (COC), and bi-directional paracrine and gap junctional communication between the oocyte and cumulus vestment are essential for oocyte viability (Eppig 1991, Albertini *et al.* 2001). The cumulus cells provide the oocyte with nutrients and regulatory signals to facilitate the progression of maturation, in particular nuclear maturation. Conversely, oocyte-secreted factors allow for cumulus cell differentiation from mural granulosa cells and mucification of the cumulus vestment (Gilchrist *et al.* 2004).

The rapid and dynamic nature of the final stages of oocyte maturation means that COCs require different compounds, such as fatty acids, amino acids, electrolytes, purines and pyrimidines and metabolites. With regard to metabolites, mature bovine COCs consume twofold more glucose, oxygen and pyruvate than immature COCs (Sutton *et al.* 2003a). A long-established tenet of COC energy metabolism is that the oocyte itself has a poor capacity to utilise glucose (Biggers *et al.* 1967), and that the cumulus cells metabolise the bulk of the glucose consumed by the COC to supply metabolic intermediates to the oocyte. The maturing COC uses glucose for energy production and numerous other

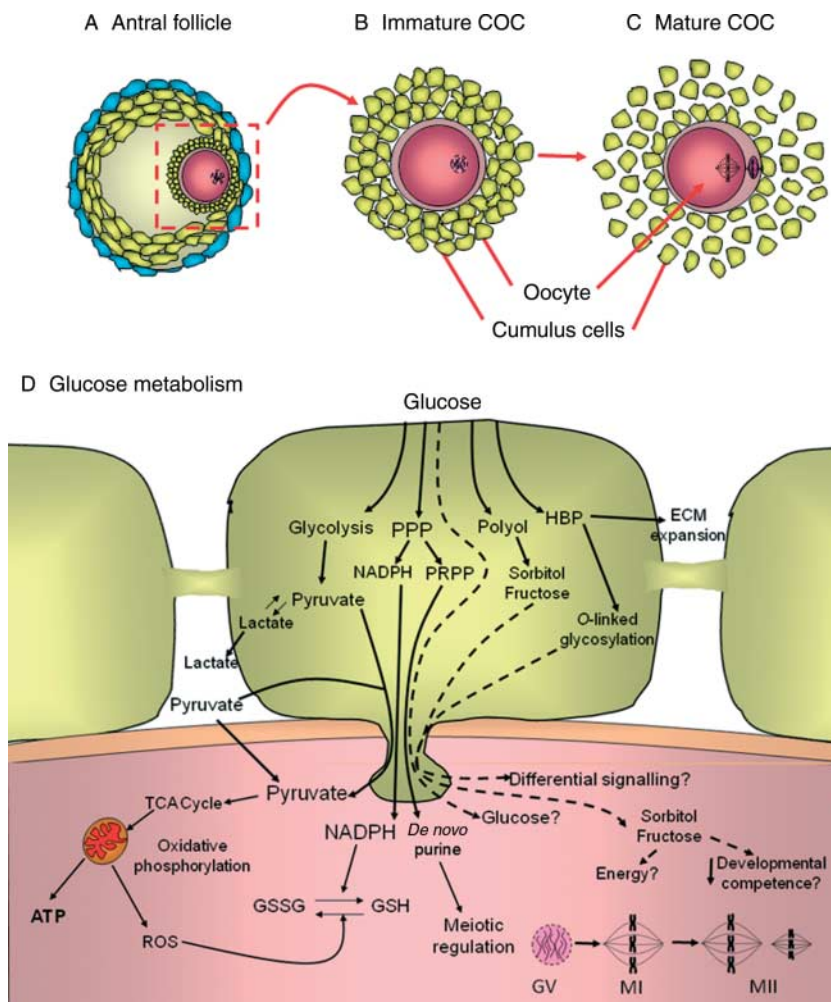


Figure 1 (A) Immature cumulus oocyte complexes (COCs; within red square) within antral follicles are characterised as having compact cumulus vestments and are arrested at prophase I (germinal vesicle stage, GV) of meiosis (B). Maturation occurs in response to gonadotrophin surges *in vivo* or release of the COC *in vitro* and is characterised by (C) expansion of the cumulus vestment and extrusion of the first polar body (metaphase II; MII). (D) Within the COC, glucose can be metabolised via four pathways. Glycolysis results in the production of pyruvate, which can be further metabolised via the tricarboxylic acid (TCA) cycle, followed by oxidative phosphorylation for energy production (ATP). The pentose phosphate pathway (PPP) produces NADPH for the reduction of the anti-oxidant glutathione (GSSG, oxidised glutathione; GSH, reduced glutathione). Phosphoribosylpyrophosphate (PRPP) is also produced by PPP and is a substrate for *de novo* purine synthesis, important for meiotic regulation within the oocyte. Products of the polyol pathway (polyol) include fructose and sorbitol. The hexosamine biosynthetic pathway (HBP) is important for producing substrates for extracellular matrices (ECM) for cumulus expansion and O-linked glycosylation (cell signalling). MI, metaphase I; ROS, reactive oxygen species.

cellular processes such as nucleic acid and purine synthesis, mucification and cellular homeostasis (Sutton *et al.* 2003b).

The environment to which the COC is exposed during maturation (both *in vivo* maturation and IVM) affects oocyte developmental competence and subsequent embryonic development. Suboptimal culture conditions during IVM result in reduced blastocyst development post-fertilisation (van de Sandt *et al.* 1990, Rose & Bavister 1992, Rose-Hellekant *et al.* 1998). Moreover, it is becoming increasingly evident that the consequences of poor maternal health, such as diabetes, obesity and poor diet, all result in hyperglycaemic increases in intra-follicular glucose levels, and that this is associated with poor oocyte viability in mice (Moley *et al.* 1998). Hence, the aim of this review is to examine the impact of glucose concentration and altering levels of glucose metabolism on oocyte and COC function. Considering the importance of the bi-directional communication between the oocyte and the cumulus vestment, the focus of this review will largely be on the metabolic activity of the COC as a whole unit. A general

overview of glucose utilisation by the COC will be discussed, followed by the roles of glucose metabolic pathways during maturation, including glycolysis, the pentose phosphate pathway (PPP), the hexosamine biosynthesis pathway (HBP) and the polyol pathway, and the consequences of maternal hyperglycaemia on oocyte development competence will be examined.

An overview of glucose metabolism

COC versus the oocyte

Prior to the ovulatory signal, the oocyte is coupled to its cumulus cells through trans-zonal processes, and this intimate physical association facilitates bi-directional communication between the oocyte and the cumulus vestment via gap junctional and paracrine signalling (Gilchrist *et al.* 2004). The oocyte relies on the cumulus vestment to facilitate the transport of glucose into the oocyte or to provide the oocyte with substrates it can utilize (Biggers *et al.* 1967). The facilitative nature of glucose transport within cumulus cells has been demonstrated by culturing immature bovine COCs in

the presence of 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-deoxyglucose (6-NBDG, Molecular Probes, Invitrogen Inc.), a fluorescent glucose analogue. Within 5–10 min of culture, 6-NBDG was present in the periphery of the cumulus vestment only (Fig. 2A) and the dispersion of 6-NBDG increased with time so that after 40 min of culture, 6-NBDG was present in all cumulus cells including the corona radiata (Fig. 2C) and was present within the oocyte by 60 min (Fig. 2D). Quantitatively, fluorescence intensity became more evenly dispersed within the COC with increasing culture time (Fig. 2E). Hence, it appears that there is a gradient through which glucose is incorporated into the cumulus

layer, with glucose taken up by cells on the outer most margin of the vestment and then, through both facilitated transport and diffusion, progresses inwards to the corona radiata and oocyte, most likely by gap junctions.

Mathematical modelling of glucose concentration gradients within the bovine COC suggests that the oocyte is exposed to glucose levels 31–82% of follicular fluid concentrations when concentrations are at physiological levels (Stokes *et al.* 2008). However, modelling shows that increases in follicular fluid glucose concentrations cause a disproportionately greater change in the concentration of glucose at the cumulus–oocyte boundary. In contrast, minimal oxygen is lost during

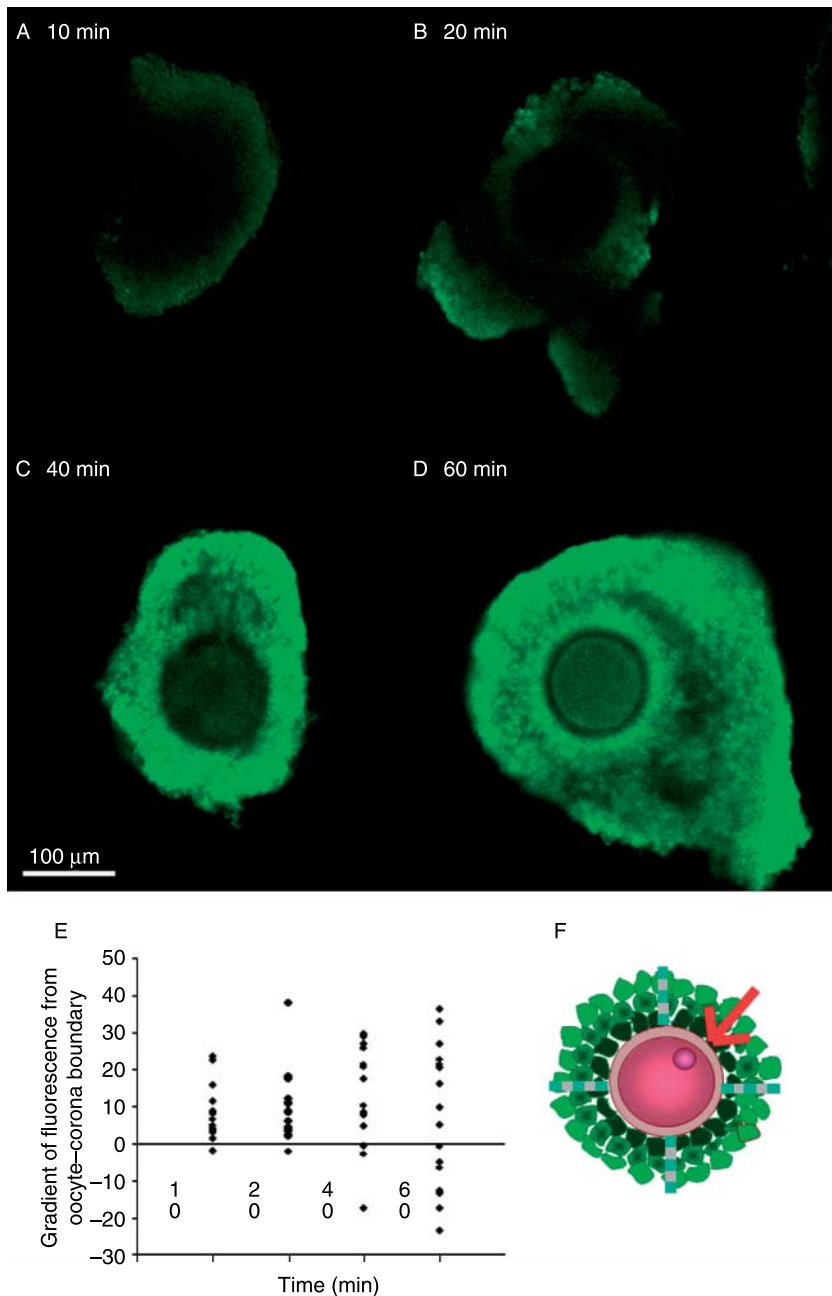


Figure 2 Incorporation of a fluorescent glucose analogue, 6-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-6-deoxyglucose (6-NBDG), into bovine cumulus oocyte complexes (COCs) over a 1-h culture period, using confocal microscopy. (A) 10, (B) 20, (C) 40 and (D) 60 min culture. (E) Gradient of fluorescence from the boundary of the oocyte and corona radiata. (F) Diagrammatic representation of the fluorescence gradient within the COC.

diffusion from the follicular fluid, through the cumulus vestment to the oocyte (Clark *et al.* 2006). Both mathematical models support the notion that glucose is preferably metabolised by the cumulus vestment to provide the oocyte with metabolites.

The oocyte itself has low capacity for glucose uptake, despite facilitative glucose transporters 1, 3 and 8 (SLC2A1, SLC2A3 and SLC2A8) expression in bovine, human, sheep and rhesus monkey oocytes (Dan-Goor *et al.* 1997, Augustin *et al.* 2001, Zheng *et al.* 2007, Pisani *et al.* 2008). In comparison, cumulus cells express an additional glucose transporter, SLC2A4 (Williams *et al.* 2001, Roberts *et al.* 2004, Nishimoto *et al.* 2006), which has a high affinity for glucose ($K_m \sim 2-5$ mM) and is an insulin-sensitive transporter, so the rate of glucose transportation into cells by SLC2A4 tends to be more reliant on insulin and insulin-like growth factors levels than on glucose concentration (Charron *et al.* 1989).

In addition to having a poor capacity to take up glucose, the bovine oocyte has low phosphofructokinase activity (PFK, one of the rate-limiting enzymes of glycolysis (Cetica *et al.* 2002)), with the consequence that the oocyte has a low glycolytic rate (Saito *et al.* 1994, Harris *et al.* 2007), and instead, relies on the cumulus cells to convert glucose to substrates it can readily utilize such as pyruvate/lactate. In fact, we have calculated that when intake of glucose and oxygen is expressed per millilitre volume of tissue per hour, cumulus cells from immature COCs consume 23-fold more glucose (oocyte = 2.2 $\mu\text{mol/ml}$ tissue per h versus cumulus cells = 50.3 $\mu\text{mol/ml}$ tissue per h) and 3.2-fold less oxygen than oocytes (oocyte = 334 $\mu\text{l/ml}$ tissue per h versus cumulus cells = 108 $\mu\text{l/ml}$ tissue per h; Thompson

et al. 2007). While this suggests that in the absence of glucose, denuded oocytes could undergo successful complete maturation if supplied with pyruvate and oxygen for energy, this is not the case as glucose is also metabolised via the PPP, HBP and polyol pathway (Fig. 3), and all these contribute to maturation and will be discussed in more detail below.

Glucose concentrations during maturation

The concentration of glucose in follicular fluid is comparable to plasma levels, ranging from 3.3 mM in humans (Leese & Lenton 1990, Gull *et al.* 1999); 1.4–5 mM in bovine (Johnson *et al.* 2001, Berg *et al.* 2003, Orsi *et al.* 2005, Sutton-McDowall *et al.* 2005); 1.2–1.7 mM in sheep (Nandi *et al.* 2007, 2008) and 0.01–2.4 mM in mouse (Harris *et al.* 2005). Follicular glucose concentrations are positively correlated with ovarian follicle size (Sutton-McDowall *et al.* 2005, Nandi *et al.* 2008). In comparison to the follicular environment where there is constant perfusion of metabolites, static IVM systems require the medium to contain a supra-physiological concentration of glucose to avoid depletion to levels detrimental to oocyte development. For example, TCM199, commonly used for bovine and ovine IVM, contains 5.6 mM glucose. Base media for mouse IVM include KSOM, α -minimum essential medium, TCM199 and Waymouth medium, with glucose concentrations ranging from 0.2 mM glucose (KSOM) to 23 mM (Waymouth medium). The supply of adequate concentrations of glucose in IVM medium leads to improved nuclear maturation and

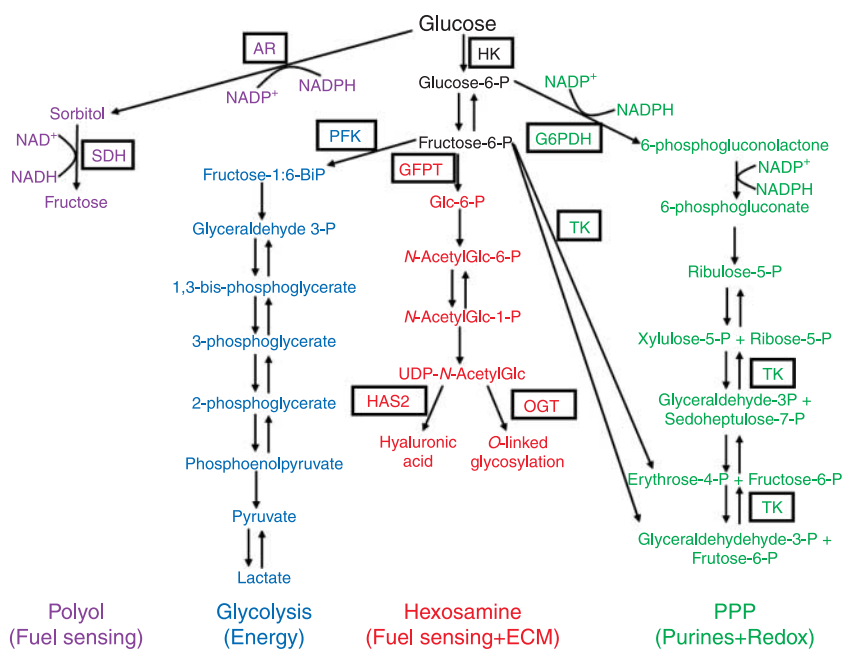


Figure 3 Metabolic pathways through which glucose can be utilised within the cumulus oocyte complex (COC). Pathways that are known to be active in the COC include the polyol pathway (purple), glycolysis (blue), hexosamine pathway (red) and pentose phosphate pathway (green). Text within boxes indicates rate-limiting or important enzymes. AR, aldose reductase; ECM, extracellular matrix; G6PDH, glucose-6-phosphate dehydrogenase; GFPT, glucosamine:fructose acetyl transferase; HAS2, hyaluronan synthase 2; HK, hexokinase; OGT, O-linked glycosylation transferase; PFK, phosphofructokinase; SDH, sorbitol dehydrogenase.

Table 1 The consequences of abnormal glucose concentrations during maturation on the developmental capacity of oocytes.

Conditions	Pathways	Outcomes	Consequences
Low glucose (0–1.5 mM)	↓ Glycolysis ↓ PPP ↓ HBP	↓ Energy availability ↓ <i>De novo</i> purine synthesis ↓ Hyaluronic acid synthesis	↓ Cytoplasmic maturation ↓ Resumption and completion of nuclear maturation ↓ Mucification Poor developmental capacity
High glucose (>10 mM)	↑ Glycolysis ↑ PPP ↑ HBP ↑ Polyol pathway	↑ Reactive oxygen species Precocious resumption of nuclear maturation Precocious <i>O</i> -linked glycosylation Precocious nuclear maturation	↓ Cytoplasmic maturation ↓ Completion of nuclear maturation (MII) ↓ Cytoplasmic maturation ↓ Nuclear maturation Poor developmental capacity

PPP, pentose phosphate pathway; HBP, hexosamine biosynthetic pathway; MII, metaphase II.

developmental capacity of oocytes (Krisher & Bavister 1998, Rose-Hellekant *et al.* 1998, Zheng *et al.* 2001). However, concentrations too low (<2.3 mM glucose) or high (>10 mM glucose) are detrimental to oocyte development (Table 1). Culturing bovine COCs in medium containing physiological or lower concentrations of glucose (1.5–2.3 mM glucose) can lead to perturbations in the completion of nuclear maturation (Sutton-McDowall *et al.* 2005) and poor embryo development post-fertilisation (Rose-Hellekant *et al.* 1998, Eppig *et al.* 2000, Ali & Sirard 2002, Ali *et al.* 2003). van de Sandt *et al.* (1990) compared the developmental outcomes of mouse COC after IVM in different base medium, with oocytes cultured in Waymouth medium having improved blastocyst development and increased cell numbers than oocytes cultured in media containing significantly lower glucose levels, seemingly contradicting the notion that high glucose levels are detrimental to oocyte developmental competence. Waymouth medium contains hypoxanthine, an inhibitor of nuclear maturation, and this may negate the negative effects of high glucose. Also, Schelbach *et al.* (2010) have shown that the COC:medium volume ratio used is critical in determining the impact of hexose concentration. A description of COC:medium volume was not published in the study of van de Sandt *et al.* (1990), but if high, this could account for the disparity. Hence, glucose availability is important in IVM but can be managed by the presence of glucose levels that allow for appropriate nuclear and cytoplasmic maturation and appropriate management of COC:medium volume ratios.

Perturbations of oocyte competence in low glucose concentration environments are thought to be manifested by a decreased flux of glucose through the PPP and through glycolysis in mice (Downs *et al.* 1998), hence limiting substrates for nucleic acid synthesis and energy production. Conversely, high glucose levels during IVM can result in increased production of reactive oxygen species, increased *O*-linked glycosylation via upregulation of the HBP and decreased concentrations of reduced glutathione (GSH), an endogenous anti-oxidant

(Hashimoto *et al.* 2000). The consequences of abnormal activity through these pathways are summarised in Table 1 and will be discussed later.

Oocyte-secreted factors and metabolism

Considering the importance of the cumulus vestment to oocyte developmental competence, the influence of oocyte-secreted factors on cumulus cell metabolic activity was examined. Glucose consumption by intact cattle COCs and oocyctomised complexes (OOX, surgical removal of the ooplasm, while retaining the cumulus vestment intact) was measured over a 24-h IVM period. Oocyte-secreted factors did not appear to affect the rate of glucose consumption, as cattle COCs, OOX and OOX co-cultured with denuded oocytes all showed similar rates of glucose consumption over a 24-h culture period (Sutton *et al.* 2003a). In contrast, mouse OOX display decreased expression of genes encoding glycolytic enzymes (including PFK, PFKP and lactate dehydrogenase, LDHA) and a tenfold decrease in glycolytic activity compared to intact COCs and OOX co-cultured with denuded oocytes (Sugiura *et al.* 2005).

There are several possible explanations for the differences in the two patterns of glucose metabolism reported between cattle and mouse cumulus cells. First, differences in glucose metabolism may be attributed to the addition of FSH to the cattle COCs and OOX cultures, whereas no hormones were added to mouse IVM system. Sugiura *et al.* (2005) suggest hormone stimulation during IVM negates the oocyte-mediated promotion of glycolysis that was seen within mouse cumulus cells. Furthermore, in the mouse study, COCs and OOX were cultured in the presence of milrinone, a modulator of nuclear maturation, hence arresting or delaying nuclear maturation. In comparison, the bovine study used a spontaneous maturation model. Alternatively, oocyte-mediated regulation of cumulus cell glycolysis may be a species-specific phenomenon, as is the case for FSH-stimulated cumulus expansion, which requires oocyte-secreted factors in the mouse (Buccione *et al.* 1990), but not in bovine and porcine COCs (Ralph *et al.* 1995, Nagyova *et al.* 1999).

Metabolic pathways important to cumulus oocyte development

The accessibility and ease of studying oocyte maturation using IVM systems have meant that the majority of studies describing oocyte glucose metabolism have utilized *in vitro* culture, rather than measurements of the metabolic activities of oocytes during *in vivo* maturation (Sutton *et al.* 2003b). Glucose consumed by the COC can be utilised for energy production, cellular homeostasis, nuclear maturation, substrates for matrices production and signalling. To date, four metabolic pathways have been identified: glycolysis (energy production), PPP, HBP and the polyol pathway (Fig. 3).

Glycolysis

The glycolytic pathway accounts for a large proportion of glucose metabolism by the COC and allows for energy production in the form of ATP and metabolites that can be readily utilised by the oocyte, such as pyruvate and lactate (Downs & Utecht 1999, Harris *et al.* 2007, 2009) and, therefore, has a fundamental role in the capacity for normal oocyte metabolism. The high capacity of cumulus cells to metabolise glucose is characterised by the presence of SLC2A1 and SLC2A4, as well as high activity of glycolytic enzymes such as 6-phosphogluconate and PFK (Downs *et al.* 1996). Once within the oocyte, pyruvate and lactate are metabolised via the tricarboxylic acid cycle, followed by oxidative phosphorylation, the predominant ATP-producing pathways within the oocyte (Steeves & Gardner 1999), while there is comparatively little glycolytic activity (Fig. 1D).

However, numerous groups have demonstrated that despite the low glycolytic activity in the oocyte, there appears to be an important positive relationship between this relatively low glycolytic activity and developmental competence of oocytes (Krisher & Bavister 1999, Spindler *et al.* 2000, Durkin *et al.* 2001, Herrick *et al.* 2006). For example, oocytes derived from pre-pubertal cattle and sheep (which have lower developmental competence) have delayed increases in glycolytic activity during maturation compared to oocytes derived from adult animals (higher developmental competence), with the rate of glucose metabolism in adult oocytes constantly increasing over a 24-h period, compared to increases in activity at the end of maturation in pre-pubertal derived oocytes (O'Brien *et al.* 1996, Steeves & Gardner 1999). Furthermore, glucose metabolism of IVM oocytes (denuded of cumulus cells prior to measurement) is lower in pig oocytes (Durkin *et al.* 2001) compared to *in vivo* matured oocytes (collected from pre-ovulatory follicles or oviductal flushing).

While the importance of the glycolytic pathway is well known, changes in the flux of glucose through this pathway during IVM have not been widely investigated.

Table 2 The consumption of glucose and production of lactate by bovine cumulus oocyte complexes during *in vitro* maturation (derived from Sutton *et al.* (2003a)). Data presented as means \pm S.E.M.

	Culture time		
	0–4 h	10–14 h	20–24 h
Glucose (pmol/ngDNA per h)	23.5 \pm 3.6	32 \pm 5.1	42.5 \pm 6.4
Lactate (pmol/ngDNA per h)	53.4 \pm 13.6	63.6 \pm 17.8	63 \pm 9.6
Glucose:lactate	2.3	1.98	1.5

n = 144 per time point.

We measured the consumption and production of metabolites by cattle COCs, and while there was a significant increase in glucose consumption over a 24-h IVM period, lactate production remained constant (Sutton *et al.* 2003a). Using the assumption that two lactate molecules are produced for one molecule of glucose consumed through the glycolytic pathway, we calculated that the flux of glucose through glycolysis remains constant during maturation (Table 2; Sutton *et al.* 2003a). The increased glucose consumed as COCs undergo maturation is accounted for by other metabolic pathways. It remains to be determined whether an altered flux of glucose through glycolysis within the COC has an effect on developmental competence.

Pentose phosphate pathway

The PPP is an important glucose metabolic pathway during maturation, and although it has never been measured within the whole COC, anecdotally only a small proportion of glucose is metabolised via this pathway as the majority of glucose consumed by the COC is metabolised by the glycolytic pathway (Downs & Utecht 1999). Likewise, in the oocyte, PPP activity accounts for <3% of the small amount of glucose metabolised by mouse oocytes (Urner & Sakkas 1999). Bovine oocytes have relatively high glucose-6-phosphate dehydrogenase (G6PDH, rate-limiting enzyme of the oxidative stage) activity compared to cumulus cells (Cetica *et al.* 2002), suggestive of higher potential PPP activity in the oocyte compared to individual cumulus cells.

The pathway can be divided into oxidative and non-oxidative stages, and glucose can enter the PPP at either stage. The oxidation of glucose-6-phosphate to 6-phosphogluconolactone by G6PDH results in the production of NADPH, and fructose-6-phosphate can also be utilised via the non-oxidative arm of the PPP by transketolase (Fig. 3). Products of the PPP include NADPH, which is utilised for cytoplasmic integrity and redox state through the reduction of glutathione (GSSG to GSH). Another product of PPP is phosphoribosylpyrophosphate, a substrate for *de novo* and salvage purine synthesis and subsequent control of nuclear maturation (Fig. 1D).

It is well established that the addition of glucose to IVM medium results in increased rates or acceleration of

nuclear maturation (Sutton-McDowall *et al.* 2005, Sato *et al.* 2007, Funahashi *et al.* 2008). However, Downs *et al.* (1996, 1998) were the first to demonstrate that the flux of glucose through PPP, rather than through glycolysis, influences the resumption of nuclear maturation in mouse COCs. Inhibition of glycolysis does not affect mouse oocyte nuclear maturation (Downs *et al.* 1996) and media containing pyruvate as the sole metabolite, result in less mouse COCs completing nuclear maturation (MII) compared to media containing glucose (Downs & Hudson 2000). Furthermore, stimulation of PPP using electron acceptors such as phenazine ethosulphate and pyrroline-5-carboxylate results in a dose-dependent increase in the rate of meiotic resumption (GV breakdown) and increased glucose metabolism (Downs *et al.* 1998).

While Downs *et al.* explored the influence of glucose and PPP activity on the resumption of meiosis, PPP is also involved in progression of all stages of meiosis in the oocyte, including the resumption of meiosis, MI–MII transition and the resumption of meiosis post-fertilisation (Sutton-McDowall *et al.* 2005, Herrick *et al.* 2006). Supplementing IVM medium with diphenyleneiodonium (inhibits NADPH oxidase) decreased PPP activity in porcine oocytes, resulting in reduced meiotic resumption and completion and decreased cleavage and blastocyst development post-fertilisation (Herrick *et al.* 2006).

Therefore, PPP activity within the oocytes is important for both nuclear and cytoplasmic maturation through the provision of substrates for purine synthesis (nuclear maturation) and intra-oocyte redox state.

Hexosamine biosynthesis pathway

Glucose can also enter the HBP, which is a major fuel-sensing pathway and is responsible for generating substrates used in matrix production (Fig. 1D). The HBP metabolises glucose-6-phosphate to fructose-6-phosphate, which is converted to glucosamine-6-phosphate by glucosamine:fructose-6-phosphate transaminase (GFPT, rate-limiting enzyme), and the end product of the pathway is UDP-*N*-acetyl glucosamine (Fig. 3). In cumulus cells, most UDP-*N*-acetyl glucosamine would be converted to hyaluronic acid by hyaluronic acid synthase 2 (HAS2). However, an alternative fate is the utilisation of UDP-*N*-acetyl glucosamine for *O*-linked glycosylation of proteins (Wells *et al.* 2003).

Cumulus expansion during oocyte maturation involves the synthesis of extracellular matrices (ECM) by cumulus cells in response to the LH surge *in vivo* and epidermal growth factor/FSH stimulation *in vitro* (Buccione *et al.* 1990). The major structural backbone of cumulus cell-derived ECM is hyaluronic acid, and both glucose and glucosamine are major substrates for hyaluronic acid synthesis (Salustri *et al.* 1989, Chen *et al.* 1990). Importantly, glucosamine is converted to

hyaluronic acid via the hexosamine pathway, but enters downstream of GFPT (Fig. 3).

As previously mentioned (section Glycolysis), ~25% of glucose consumed by bovine COCs in the later period of IVM is not metabolised via glycolysis (Sutton *et al.* 2003b). Supplementing IVM with glucosamine resulted in a significant decrease in glucose consumption and less incorporation of radio-labelled glucose in the ECM of COCs after FSH stimulation (Sutton-McDowall *et al.* 2004), indicating a flux of glucose through the HBP. Furthermore, inhibition of HBP using the GFPT inhibitor 6-diazo-5-oxo-L-norleucine results in decreased cumulus expansion and glucose uptake by COCs (Gutnisky *et al.* 2007). Hence, the increased rate of glucose consumption by COCs towards the end of IVM is to generate matrix via the HBP.

While cumulus expansion is commonly associated with improved developmental competence, for example, HAS2 expression in cumulus cells is associated with increased oocyte developmental competence (Assidi *et al.* 2008), increased activity of the HBP can have deleterious effects on oocyte health. In somatic cells, HBP is involved in fuel sensing and under euglycaemic conditions accounts for 1–3% of total glucose metabolism (Marshall *et al.* 1991). The fuel-sensing role of HBP appears to be mediated by *O*-linked glycosylation of proteins. There is a close relationship between phosphorylation and *O*-linked glycosylation. Serines and threonines can be targets of *O*-linked glycosylation and some can be either glycosylated or phosphorylated, resulting in upregulation or downregulation of protein signalling pathways (Wells *et al.* 2003). Increased flux of glucose through the HBP leads to increased *O*-linked glycosylation, leading to changes in the target protein conformation and subsequent in/activation of downstream targets. Indeed, increased activity of the HBP is recognised as one of the contributing factors of the diabetic pathology in human somatic cells (Brownlee 2001).

The influence of hyperglycaemic conditions on the activity of the HBP within the COC has not been determined. However, glucosamine is commonly used in somatic cell cultures to upregulate HBP activity as glucosamine enters downstream from GFPT, hence mimicking hyperglycaemic environments (Andreozzi *et al.* 2004). Glucosamine supplementation during IVM does not affect nuclear maturation or cleavage rates post-fertilisation in cattle, pig and mouse oocytes (Sutton-McDowall *et al.* 2006, Schelbach *et al.* 2010). However, glucosamine supplementation during IVM severely perturbed post-compaction embryo development, most likely by increasing *O*-linked glycosylation in cumulus cells (Sutton-McDowall *et al.* 2006, Schelbach *et al.* 2010). The pathways targeted by *O*-linked glycosylation within cumulus cells are yet to be determined.

Polyol pathway

The polyol pathway involves the oxidation of glucose to sorbitol and fructose by aldose reductase (AR) and sorbitol dehydrogenase (SDH). Under normal glycaemic conditions, the polyol pathway accounts for very little of total glucose metabolism by somatic cells, largely due to AR having a low affinity for glucose compared to other enzymes, such as hexokinase. However, in a hyperglycaemic environment, the flux of glucose through the polyol pathway can increase to between 11 and 33% (human erythrocytes and eye lens respectively) as the hexokinase enzyme becomes saturated. Increased activity of the polyol pathway is thought to manifest diabetic pathology through numerous different mechanisms, including intracytoplasmic accumulation of sorbitol and fructose, both of which have poor membrane permeability; decreased levels of NADPH (a co-factor of AR); pseudo-hypoxia by increasing NADH/NAD⁺ (NAD⁺ is a co-factor of SDH) or altering lactate/pyruvate ratios mediated by SDH and activation of protein kinase C (Brownlee 2001).

Under normal glycaemic conditions, SDH and AR are expressed in reproductive tissues, and the highest levels of protein and enzymatic activity are found in the ovary (Kaneko *et al.* 2003). AR is expressed in rat granulosa cells and oocytes, and SDH is highly expressed in the oocyte (Iwata *et al.* 1990, Kaneko *et al.* 2003). While cumulus cells have high levels of glycolytic activity to provide the oocyte with metabolites for oxidative phosphorylation (Sutton *et al.* 2003b), localisation of both enzymes suggests that granulosa cells convert glucose to sorbitol, providing the oocyte with alternative substrates for energy production, namely fructose. To date, sorbitol and fructose levels within the oocyte or COC have not yet been measured. However, when fructose is the sole hexose source during IVM, significantly less oocytes complete nuclear maturation compared to COCs cultured in the presence of glucose (Wongsrikeao *et al.* 2006), supporting the requirement of glucose for nuclear maturation.

Limited data are available about the roles of SDH and AR during oocyte maturation. Within the ovary AR mRNA levels in granulosa cells increase in the absence of oestrogen and during follicle atresia (Svanberg *et al.* 2000), suggesting the polyol pathway is involved in ovarian tissue differentiation and remodelling during the oestrous cycle.

Maternal environment and oocyte developmental competence

It is well established that poor maternal health, in particular maternal hyperglycaemia induced by type I diabetes (hypoinsulinaemia), type II diabetes, poor diet or obesity, results in reduced fecundity, increased miscarriage rates and increased risk of congenital

abnormalities (Moley *et al.* 1998). However, it is becoming increasingly evident that the environment during the peri-conception period is important for long-term programming of offspring (Doblado & Moley 2007).

The effect of maternal type I diabetes (and associated hyperglycaemia) on oocyte maturation has been largely studied in either transgenic or chemically-induced diabetic rodent models. In comparison, there is currently limited data on the influences of maternal type II diabetes on oocyte developmental competence, and this is surprising considering the increased incidence in western societies. Hence, the effects of maternal hyperglycaemia on oocyte developmental competence will be discussed using the type I diabetes model. Hyperglycaemia is defined as fasting blood glucose levels of 240–300 mg/dl or greater (13.3–16.7 mM glucose) and can be induced in mice by streptozotocin-mediated destruction of pancreatic β -cells (Like & Rossini 1976).

Oocytes derived from mice with type I diabetes and hyperglycaemia have poor developmental competence due to poor folliculogenesis, oogenesis and oocyte maturation. Induction of diabetes in mice results in perturbed folliculogenesis and increases the incidence of follicular apoptosis, hence reducing ovulation rates (Chang *et al.* 2005). In addition, mouse oocytes from pre-ovulatory follicles are ~30% smaller in size compared to oocytes from control animals (Chang *et al.* 2005). Secondly, COCs from chemically induced diabetic mice exhibit aberrant nuclear maturation, with precocious resumption of meiosis during spontaneous maturation and decreased efficiency of induced maturation, leading to an MI–MII transition block in both models (Kim *et al.* 2007). Poor nuclear maturation was attributed to decreased *de novo* purine and cAMP synthesis as a result of decreased flux of glucose through PPP (Colton *et al.* 2003). Interestingly, the flux of glucose through glycolysis was not affected (Colton *et al.* 2003), with excess glucose shunted through the polyol pathway (Colton & Downs 2004).

Although Colton *et al.* (2003) reported no alterations in the flux of glucose through the glycolytic pathway, another study reported increased AMP:ATP ratios in denuded oocytes from diabetic mice (Ratchford *et al.* 2007). Increased AMP:ATP ratios indicate a rapid use of intra cellular ATP and is associated with a response to stress. It is likely that AMP-dependent kinases are involved, which have been associated with altered developmental competence cow, mouse and pig oocytes (Richard *et al.* 1997, Downs *et al.* 2002, Downs & Chen 2006, Mayes *et al.* 2007). Furthermore, it was suggested that glycolytic activity was blocked, indicated by the accumulation of fructose bisphosphate and low ATP levels (Ratchford *et al.* 2007).

Poor oocyte growth, meiotic competence and glucose metabolism within COCs derived from diabetic mice may also be attributed to decreased gene and protein

expression of connexins, the structural units making up gap junction channels, resulting in 60% less gap-junction communication between the oocyte and cumulus vestment (Ratchford *et al.* 2008). This is significant considering the reliance of the oocyte on cumulus cell communication for developmental competence.

While there are very few studies relating obesity, hyperinsulinaemia and hyperglycaemia during the peri-conception period and human oocyte developmental competence, the follicular fluid composition of patients undergoing assisted reproduction with different body mass indexes (BMIs, kg/m²) was analysed and compared to oocyte collection, embryo development and pregnancy rates (Robker *et al.* 2009). Oocyte quality was significantly affected by increasing BMI with less oocytes collected and less embryos produced from obese patients (BMI ≥ 30). Increasing BMI also resulted in alterations in follicular fluid composition, in particular increases in follicular insulin, glucose and lactate concentrations (Robker *et al.* 2009). Considering one of the main glucose transporters expressed in cumulus cells is the insulin sensitive SLC2A4 (section COC versus the oocyte), oocytes derived from obese women (BMI ≥ 30) are likely to be exposed to significantly higher glucose levels through a combination of higher follicular glucose levels and increased activity of glucose transporters within cumulus cells. Hence, exposure to high glucose levels may be a major contributor to poor oocyte quality in obese women. Glucose:lactate ratios were not altered between the different groups, indicating that glycolytic activity was not affected (Robker *et al.* 2009). Therefore, it is highly likely that excess follicular glucose may be directed through the hexosamine biosynthesis and polyol pathways, both fuel-sensing pathways that have negative consequences on oocyte competence.

Conclusions

While the notion that maternal health during pregnancy has an impact on long-term health of offspring is well established (Barker 2004), it is becoming increasingly evident that the peri-conceptual period is also important. Glucose is significant in every aspect of final oocyte maturation, as demonstrated by its effects on meiotic, cytoplasmic and cumulus cell maturation. Therefore, alterations in glucose metabolism are likely to be a cause for decreased oocyte competence and reduced fecundity.

Within the COC, glucose is metabolised via four main pathways, and substrates of these pathways affect oocyte cytoplasmic and nuclear maturation (Fig. 1D). As the oocyte itself is exposed to lower glucose levels compared to the follicular fluid concentrations, most likely differential regulation of metabolic pathways and products of glucose metabolism affect developmental competence, rather than simply glucose alone.

Further understanding of glucose metabolism during oocyte maturation may lead to the development of improved IVM culture conditions, as well as intervention plans and treatment for women with low fertility who are obese, insulin resistant or diabetic.

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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