Culture environment modulates maturation and metabolism of human oocytes

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BACKGROUND: The clinical use of oocytes matured *in vitro* for IVF is increasing, but little is known about the effect of culture conditions on oocyte maturation. METHODS: Denuded immature oocytes identified following superovulation prior to ICSI were individually matured in one of two commercial media: tissue culture medium (TCM) 199 or modified Eagle medium with Earle's modified salts (MEME). During maturation, depletion of pyruvate and accumulation of lactate in the culture medium were non-invasively measured. RESULTS: Maturing oocytes took up pyruvate (20–30 pmol/oocyte/h) and produced lactate (2–10 pmol/oocyte/h). Oocytes matured faster in MEME, with significantly more oocytes reaching metaphase II by 24 h after oocyte retrieval compared with TCM 199 ($P = 0.03$). The oocytes that matured more quickly in MEME had significantly lower lactate production than oocytes that matured more slowly ($P = 0.02$). In TCM 199, pyruvate uptake by rapidly maturing oocytes was lower than by slowly maturing oocytes ($P = 0.05$). During the second incubation, from 24 to 48 h post-oocyte retrieval, pyruvate uptake in MEME was 30% lower than in TCM 199 ($P = 0.007$). Pyruvate uptake and lactate production differed depending on the stage of nuclear maturation: pyruvate uptake and lactate production were greater during germinal vesicle breakdown than during polar body extrusion in MEME ($P < 0.05$). CONCLUSIONS: We have shown that: (i) pyruvate is a major energy source during oocyte maturation; (ii) the composition of the culture medium can affect the rate of maturation; and (iii) the culture medium and stage of nuclear maturation can affect pyruvate uptake and lactate production.

Key words: IVF/IVM/lactate/medium/pyruvate

Introduction

In-vitro maturation (IVM) of oocytes from small antral follicles could potentially provide increased numbers of oocytes for IVF, reducing the need for exogenous gonadotrophin treatment and offering an alternative to superovulation. In humans there are several such follicles present in the early follicular phase, the majority of which regress by atresia in the few days before ovulation. Pregnancies and live births have been achieved following maturation and fertilization *in vitro* of oocytes retrieved from small antral follicles (Trounson *et al.*, 2001), but clinical pregnancy rates are generally lower than those achieved using standard IVF.

A suboptimal environment for maturation *in vitro* is one of many factors that could account for the low pregnancy rates. Studies in cows and mice have shown that a variety of commercially available culture media have widely differing effects on spontaneous nuclear maturation and subsequent preimplantation development following IVF (van de Sandt *et al.*, 1990; Rose and Bavister, 1992). Indeed, in domestic species, the exposure of oocytes and early embryos to certain culture conditions can have profoundly deleterious effects on

preimplantation, fetal and post-natal development, resulting in a spectrum of abnormalities collectively referred to as 'large offspring syndrome' (Young *et al.*, 1998; McEvoy *et al.*, 2001). It has been proposed that genesis of these abnormalities may involve cellular stress responses to culture conditions and, consequently, disturbances in metabolism and gene expression which result in abnormal development (Leese *et al.*, 1998). In preimplantation embryos, gene expression and genomic imprinting is altered under different culture conditions (Ho *et al.*, 1995; Doherty *et al.*, 2000; Niemann and Wrenzycki, 2000; Khosla *et al.*, 2001). A wide variety of culture media, which may be supplemented with any of a number of gonadotrophins, steroids and growth factors, have been used for the maturation of human oocytes *in vitro* before fertilization and embryo transfer (Trounson *et al.*, 2001). The animal studies described above highlight the need for a greater understanding of both human oocyte maturation and the effects of the maturation environment on subsequent embryo and fetal development.

In vivo, oocytes resume meiosis and undergo nuclear maturation following the LH surge, although fully grown oocytes from many species can mature spontaneously, resuming

meiosis following release from the follicle (Pincus and Enzmann, 1935; Edwards, 1965). A critical part of the maturation process may involve the development and activation of appropriate metabolic pathways as metabolism shifts from involving both granulosa cells and the oocyte within the cumulus–oocyte complex to being oocyte-centred. This shift is important in ensuring the metabolic independence of the oocyte following ovulation and loss of gap junctional communication with surrounding cumulus cells.

Despite increased clinical interest in human IVM over the past decade, little is known about the metabolism of the maturing oocyte, particularly in the absence of surrounding cumulus cells. Pyruvate is produced by cumulus cells (Leese and Barton, 1985), and is the major energy source for maturing mouse, cow and cat oocytes (Biggers et al., 1967; Steeves and Gardner, 1999a; Khurana and Niemann, 2000), with minimal metabolism of glucose (Rieger and Loskutoff, 1994; Spindler et al., 2000). Energy substrates have also been shown to play an important role in modulating oocyte maturation by interacting with meiotic inhibitors such as hypoxanthine (Fagbohun and Downs, 1992; Downs and Mastropolo, 1994).

Superovulation techniques aim to maximize oocyte maturation in vivo; however, 5–15% of aspirated oocytes remain at the germinal vesicle (GV) stage at the time of oocyte collection (Janssenswillen et al., 1995; Farhi et al., 1997). Why these oocytes fail to complete nuclear maturation remains unknown; the follicles may be resistant, or less responsive, to hormonal stimulation (Farhi et al., 1997), a proportion may be atretic (Goud et al., 1998) or they may simply be at an earlier developmental stage (Kim et al., 2000). The latter is the most likely scenario as, with further culture, a cohort will complete nuclear maturation, and can be successfully fertilized and will initiate embryonic development (Janssenswillen et al., 1995; Farhi et al., 1997; Goud et al., 1998; Haberle et al., 1999; Kim et al., 2000). Pregnancies and live births have been achieved using such in-vitro matured oocytes (Nagy et al., 1996; Edirisinghe et al., 1997; Tucker et al., 1998). Oocytes that remain immature at the time of oocyte collection are not suitable for ICSI. The exposure of these oocytes to hCG before oocyte retrieval, followed by the removal of cumulus cells to confirm maturity prior to ICSI, will promote resumption of meiosis. These denuded oocytes can be used to study the effect of culture media on the completion of oocyte maturation and the accompanying changes in metabolic requirements.

The aims of this study were to: (i) evaluate whether the basic culture medium alone, in the absence of hormones and growth factors, could influence rates of human oocyte maturation; (ii) assess non-invasively, for the first time, pyruvate uptake and lactate production by oocytes as they complete nuclear maturation in vitro; and (iii) ascertain whether the composition of the culture medium affects oocyte metabolism. To achieve these aims, we matured individual GV stage oocytes in one of two commercial culture media commonly used for oocyte maturation: tissue culture medium (TCM) 199 and modified Eagle medium with Earle's modified salts (MEME). During maturation we non-invasively measured the depletion of pyruvate and the accumulation of lactate in the culture medium.

Materials and methods

Source of human oocytes

Immature oocytes were donated to research, with informed consent, from 44 women undergoing cycles of ICSI. Following pituitary desensitization with a GnRH analogue (Buserelin; Hoechst, Middlesex, UK), the women underwent ovarian stimulation using recombinant FSH (Puregon; Organon, Cambridge UK, or Gonal F; Serono, Welwyn Garden City, UK). When the ovaries contained three or more follicles >17 mm in diameter and the estradiol levels were >3500 pmol/l, 10 000 IU of hCG (Profasi; Serono) was administered and transvaginal oocyte retrieval took place 36–38 h later. Oocytes were denuded of cumulus cells by repeated pipetting in an 80 IU/ml hyaluronidase solution (Sigma, Poole, UK) and metaphase II oocytes were selected for ICSI. Sibling and single immature oocytes that were still at the GV stage were initially cultured either in Earle's Balanced Salt Solution (Life Technologies, Paisley, UK) (Dawson et al., 1995), Sydney IVF Fertilization medium (Cook UK Ltd, Letchworth, Herts, UK) or Universal IVF medium (Medicult UK Ltd, Redhill, Surrey, UK). These oocytes were donated to research on the afternoon of oocyte retrieval (day 0) and sibling oocytes were randomly allocated to one of two experimental media (see below). Only morphologically normal oocytes were included in the study. Oocytes with uneven, granular cytoplasm, cytoplasmic vacuoles or evidence of degeneration were excluded. Oocytes were classified as being at: (i) GV stage if the nuclear membrane remained intact and was clearly visible by light microscopy; (ii) metaphase I if there was no visible nuclear membrane; or (iii) metaphase II following extrusion of the first polar body.

IVM

Two commercially available media were used for further culture of the immature oocytes, TCM 199 (Gibco BRL, Paisley, UK) and MEME (Gibco) (Table I). Both media contain inorganic salts, glucose and essential amino acids and are supplemented with 0.4% (v/v) human serum albumin (Zenalb 20; Bio Products Laboratory, Elstree, Herts, UK), 0.47 mmol/l sodium pyruvate (Sigma), 83.2 IU/ml penicillin (Sigma) and 0.075 mg/ml streptomycin. TCM 199 is more complex than MEME, containing, in addition, DNA and RNA precursors and a wider range of non-essential amino acids and vitamins.

Denuded sibling oocytes were randomly and evenly divided between the two culture conditions; single oocytes were randomly distributed to either medium. Oocytes were cultured individually in pre-equilibrated drops of medium overlaid with silicone fluid (Dow Corning 200/50 cs; BDH) in an atmosphere of 5% CO_2 in air at 37°C. Initially, oocytes were cultured in drops of ~20 μl ($n = 19$) until 48 h post-oocyte retrieval. Subsequently oocytes ($n = 59$) were washed through 3 drops of culture medium before culture in 5 μl drops for analysis of pyruvate uptake and lactate production. Drops were laid down using a positive displacement pipette (SMI digital adjust micro/pettor, accuracy: ±1%; Alpha Laboratories, Eastleigh, Hants, UK). Oocytes were placed in culture on the afternoon of the day of oocyte retrieval, and oocyte maturation was assessed immediately and then again on the mornings of day 1 (24 h post-oocyte retrieval) and day 2 (48 h post-oocyte retrieval). On the morning of day 1, oocytes were moved from the incubation drops to fresh 5 μl incubation drops for culture until the morning of day 2. Oocytes were also photographed at these times.

Measurement of pyruvate uptake and lactate production

Fifty-nine immature ICSI oocytes (which had been completely denuded of cumulus cells) were cultured singly in 5 μl drops of

Table I. Composition of TCM 199 and MEME

	Component	TCM 199	MEME
Inorganic salts	e.g. NaCl, KCl, NaHCO$_3$, CaCl$_2$, MgSO$_4$	+	+
DNA/RNA precursors	Purines/pyrimidines	+	–
	Nucleoside esters	+	–
	Purine metabolites	+	–
	Cholesterol	+	–
Sugars	Glucose	5.6 mmol/l	25 mmol/l
	Lactate	–	–
	Pyruvate[a]	0.47 mmol/l	0.47 mmol/l
	Ribose	+	–
Detergent	Tween 80	+	–
Antioxidant	Glutathione	+	–
Amino acids	Essential	All	All
	Non-essential	All	Cys, Tyr
	Glutamine	0.68 mmol/l	3 mmol/l
Vitamins		+ +	+
Protein	Human serum albumin[a] (0.4% v/v)	+	+

[a]Pyruvate and human serum albumin were added fresh separately, just prior to incubation of oocytes.

either TCM 199 ($n = 26$) or MEME ($n = 33$) overnight from day 0 to day 1 (~17 h), and for a further 24 h in fresh 5 μl drops of the same medium. Similar 5 μl drops of medium alone were incubated alongside the oocyte-containing drops and served as controls. At the end of each culture period, individual 3 μl aliquots of the spent and control drops from the recently terminated culture dishes were diluted with 597 μl of a 5 μmol/l lactate solution (lactate standard; Sigma). Pyruvate uptake and lactate production were measured by analysing the difference between nutrient concentrations in the control and incubation drops as described previously (Hardy *et al.*, 1995). The high concentration of glucose in these commercial media precluded the accurate measurement of glucose uptake by single oocytes.

Ethical approval

The study was granted ethical approval by the Research Ethics Committee for Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals, and written consent was obtained from all participating patients.

Statistical analysis

χ^2 and Mann–Whitney U-tests were used for statistical analyses as appropriate. Significance was accepted when $P < 0.05$.

Results

Source of oocytes

A total of 636 oocytes were collected from 44 women undergoing a cycle of ICSI; of these, 466 (73.3%) were at the metaphase II stage, 40 (6.3%) at metaphase I, 100 (15.7%) at GV and 30 (4.7%) were degenerate. One hundred denuded immature ICSI oocytes were donated to research, of which 78 were suitable for further maturation *in vitro* on the basis of the morphological criteria described above. A total of 36 were cultured in TCM 199 and 42 in MEME. Pyruvate uptake and lactate production by 59 of these oocytes were assessed (26 in TCM 199 and 33 in MEME). Five oocytes with persistently adherent cumulus cells were excluded from further analysis. In allocating oocytes to each of the two culture media, there was no statistical difference in patient population with regard
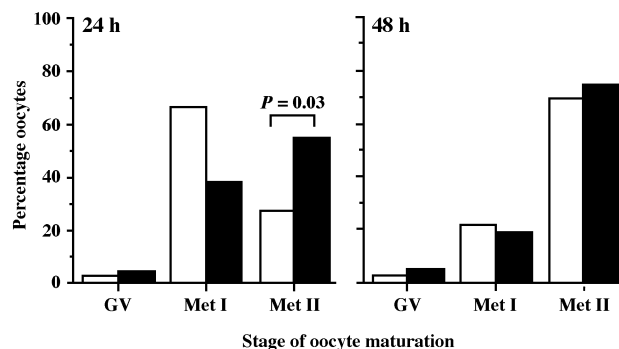

Figure 1. Proportion of oocytes at the germinal vesicle (GV), metaphase I (Met I) and metaphase II (Met II) stages of maturation at 24 h (left panel) and 48 h (right panel) post-oocyte retrieval, after culture in TCM 199 (□, $n = 36$) and MEME (■, $n = 42$).

to age, peak estradiol levels, follicle diameters at the time of oocyte collection, number of oocytes retrieved and oocyte nuclear stage at the time of aspiration.

IVM

All oocytes were at the GV stage at the time of oocyte retrieval. Those donated to research were available for study on the afternoon of oocyte retrieval, by which time a proportion had already initiated GV breakdown (GVBD). Denuded oocytes were randomly allocated to the two culture media before scoring and retrospective analysis showed that there was no significant difference between the two groups in terms of developmental stage at 6 h. At 24 h post-aspiration, significantly more oocytes had progressed to metaphase II in MEME (55%) than in TCM 199 (28%) ($P = 0.03$; Figure 1). At 48 h post-aspiration, the majority of oocytes had completed nuclear maturation with 74% metaphase II oocytes in MEME compared with 69% in TCM 199 (not significant; Figure 1).

Pyruvate uptake and lactate production

Pyruvate uptake and lactate production by individual denuded ICSI oocytes were measured during two successive incubations
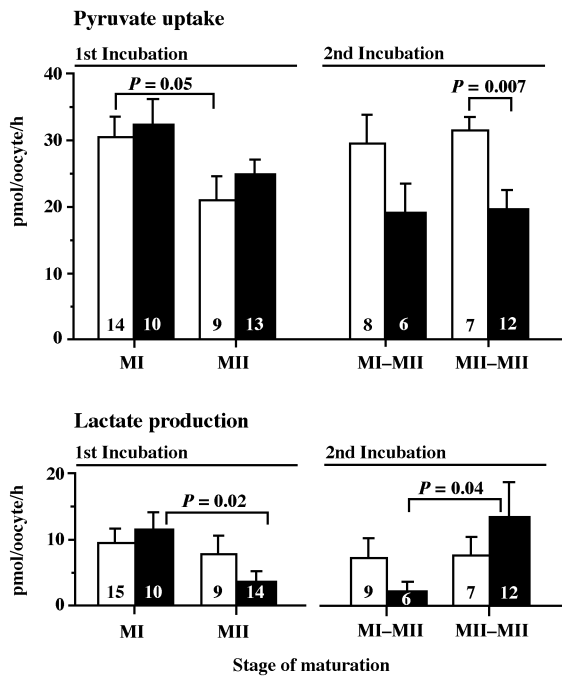
Figure 2. Pyruvate uptake and lactate production (pmol/oocyte/h) by oocytes at metaphase I (Met I) and metaphase II (Met II) stages at the end of the first incubation period (left panel); and by oocytes maturing from metaphase I to metaphase II (Met I–Met II) or remaining at the metaphase II stage (Met II–Met II) during the second incubation period (right panel) in TCM 199 (□) and MEME (■). Numbers in each bar = number of oocytes analysed.

Figure 3. Effect of stage of nuclear maturation on pyruvate uptake (left, pmol/oocyte/h) or lactate production (right) by oocytes cultured in TCM 199 (□) or MEME (■). GVBD = germinal vesicle breakdown; pb ext. = polar body extrusion; Met II–Met II = oocytes which remain at the metaphase II stage during a single incubation period (*$P < 0.05$). Numbers in each bar = number of oocytes analysed.

over a 48 h period (Figure 2). During the first incubation, some oocytes completed maturation (fast maturers), while some only reached metaphase I (slow maturers) during this initial incubation. In TCM 199, pyruvate uptake for fast maturers was lower than for slow maturers ($P = 0.05$) (Figure 2).

Comparing the effects of different media, it was found that during the first incubation, pyruvate uptake was similar in TCM 199 and MEME (Figure 2). In contrast, during the second incubation pyruvate uptake was higher in TCM 199 than in MEME, significantly so in fast maturers which had reached metaphase II during the previous incubation period ($P = 0.007$) (Figure 2). Oocytes that failed to mature continued to take up pyruvate and produce lactate at levels similar to oocytes which matured slowly (data not shown).

Lactate production by individual oocytes was lower than pyruvate uptake (Figure 2). In MEME, lactate production during the first incubation was significantly lower for fast maturers than for slow maturers, i.e. oocytes which had only reached metaphase I ($P = 0.02$). During the second incubation period, lactate production was significantly lower for slow than for fast maturers ($P = 0.04$). In TCM 199, lactate production was similar in the first and second maturation period, irrespective of whether oocytes were maturing quickly or slowly.

By assessing the maturational stage of the oocyte at the beginning and end of each incubation period, it was possible to correlate substrate uptake/production with a particular cellular event occurring during maturation. Pyruvate uptake was less
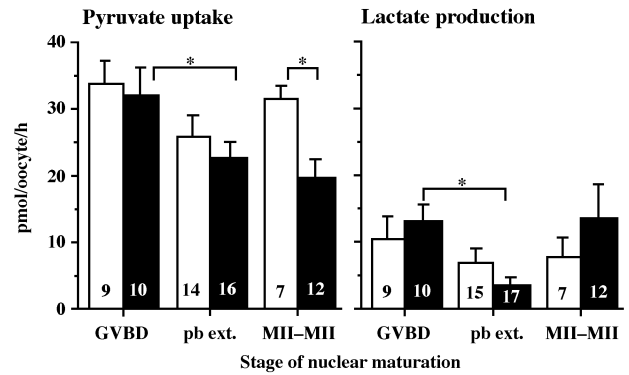
during polar body extrusion than GVBD in both media, significantly so in MEME ($P < 0.05$, Figure 3). There was no significant difference in pyruvate uptake between the two media during GVBD or polar body extrusion (Figure 3). However, when oocytes that had already completed maturation and extruded their first polar body during the first culture period were cultured for a further 24 h, pyruvate uptake was significantly greater for those in TCM 199 than in MEME ($P < 0.01$, Figure 3). In TCM 199 there was no significant difference in the amount of lactate produced during GVBD, polar body extrusion or for oocytes remaining at metaphase II. However, when oocytes were matured in MEME, significantly less lactate was produced during polar body extrusion than during GVBD ($P < 0.01$, Figure 3).

The possibility of using substrate uptake as a predictor of an oocyte's competence to reach complete maturation was also evaluated. The metabolism during the first culture period of oocytes that completed maturation during the subsequent culture period was compared with that of oocytes that arrested at metaphase I. As there was no significant difference in the metabolism of oocytes undergoing GVBD and polar body extrusion under the two culture conditions (Figure 3), data were combined. Pyruvate uptake was similar for those oocytes that went on to complete maturation and those that arrested: 35.4 ± 2.6 ($n = 7$) versus 33.1 ± 2.3 ($n = 6$) pmol/oocyte/h respectively. Lactate production was lower for oocytes that went on to complete maturation during the second culture period: 4.7 ± 2.3 versus 12.1 ± 3.5 pmol/oocyte/h, but this difference was not statistically significant.

Discussion

This study is the first description of the non-invasive measurement of pyruvate uptake and lactate production by individual denuded human oocytes as they undergo nuclear maturation. Here we have shown, for the first time, that exposure of maturing human oocytes to two different commonly used commercial culture media (TCM 199 and MEME) for 48 h affects oocyte metabolism, specifically pyruvate uptake. More

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oocytes reached metaphase II during the first culture period in MEME compared with TCM 199, and the oocytes that matured more quickly had significantly lower lactate production than more slowly maturing oocytes. In TCM 199, rapidly maturing oocytes also had lower pyruvate uptake than slowly maturing oocytes. Pyruvate uptake and lactate production differed depending on the stage of nuclear maturation: pyruvate uptake and lactate production were greater during GVBD than polar body extrusion in MEME.

The maturation rates seen over a 48 h period are comparable with those achieved by others working with denuded, immature oocytes retrieved from women following superovulation (Janssenswillen *et al.*, 1995; Farhi *et al.*, 1997; Goud *et al.*, 1998; Haberle *et al.*, 1999). Significantly more oocytes reached metaphase II within 24 h in MEME than in TCM 199 (Figure 1). This could be important clinically, as earlier first polar body extrusion by mouse oocytes has been associated with improved developmental competence (van de Sandt *et al.*, 1990). In general terms, MEME is a less complex medium than TCM 199. Both contain a similar range of inorganic salts, but MEME has a higher glucose concentration, fewer non-essential amino acids and a relatively small number of vitamins (Table I). TCM 199 has a lower glucose concentration, both essential and non-essential amino acids, a larger range of vitamins, purines and pyrimidines, and several other components such as cholesterol, glutathione and ribose (Table I). Amino acids are known to be beneficial for embryo development (Gardner and Lane, 1993; Steeves and Gardner, 1999b; Devreker *et al.*, 2001) and oocyte maturation (Rose-Hellekant *et al.*, 1998; Watson *et al.*, 2000) in several species. However, certain amino acids are known to have a detrimental effect on hamster embryo development (Bavister and McKiernan, 1993). The different amino acid content between the two culture media may account for the difference in maturation rates seen in this study.

Other candidate factors for the relative inhibitory effect of TCM 199 on maturation of denuded immature ICSI oocytes are purines and purine precursors, both of which are present in this medium (Table I). Extensive studies on the maturation of cumulus-enclosed and denuded mouse oocytes have established the existence of a wide range of meiosis-inhibiting factors, including purines such as hypoxanthine (Downs *et al.*, 1985), nucleosides such as adenosine (Eppig *et al.*, 1985), and purine precursors such as glutamine, glycine and aspartic acid (Downs, 1998). The maturation-arresting action of these factors involve complex interactions between components in the media (for example glucose and pyruvate) and the oocyte and its companion cumulus cells. In the present study, several meiosis-arresting substrates, or their precursors, are present in TCM 199 that are not included in MEME, namely hypoxanthine, AMP, aspartic acid and glycine, which may explain the slower rate of maturation. However, levels of hypoxanthine in TCM 199 (2.2 µmol/l) are significantly lower those used to maintain meiotic arrest of denuded mouse oocytes (4 mmol/l) (Downs and Mastropolo, 1994), and such hypoxanthine-induced arrest can be completely reversed in the presence of 0.5 mmol/l pyruvate (which is also present in TCM 199). It is therefore unlikely that hypoxanthine *per se* is inhibiting maturation,

although this does not preclude the possibility that the purines and purine precursors present in combination in TCM 199 may slow the rate of maturation.

Studies examining the metabolism of radiolabelled energy substrates during maturation of cumulus-free cow and cat oocytes (Rieger and Loskutoff, 1994; Steeves and Gardner, 1999a; Spindler *et al.*, 2000) have shown that pyruvate is the predominant energy source. We have shown here that pyruvate uptake by maturing human oocytes is also high (Figures 2 and 3). Furthermore, levels of pyruvate uptake and lactate production by human oocytes maturing *in vitro* are consistent with previous studies in human embryos. The amount of pyruvate taken up throughout maturation is of a similar order of magnitude to that taken up by human oocytes (Leese *et al.*, 1986) and embryos during their first cleavage divisions (Hardy *et al.*, 1989; Gott *et al.*, 1990; Conaghan *et al.*, 1993; Turner *et al.*, 1994; Devreker *et al.*, 1998, 1999), but is higher than that of fertilized human zygotes following ICSI (Devreker *et al.*, 2000).

Lactate was produced by maturing human oocytes *in vitro* at rates similar to those described previously for early human preimplantation embryos (Hardy *et al.*, 1995; Devreker *et al.*, 1998) but lower than that seen by Gott *et al.* (Gott *et al.*, 1990). It has previously been proposed that lactate produced by preimplantation mouse and human embryos is derived from exogenous glucose (Gott *et al.*, 1990). Levels of glucose in the two commercial media used here were high (Table I) compared with levels measured in fluid from the human Fallopian tube and uterus (Gardner *et al.*, 1996), and glucose uptake by oocytes is negligible (Devreker *et al.*, 2000). Studies using radiolabelled pyruvate as the sole energy source for 2-cell mouse embryos *in vitro* have demonstrated that lactate can be produced from pyruvate (Wales and Whittingham, 1970). Culture of early human embryos in medium containing pyruvate alone (Butcher *et al.*, 1998) resulted in lactate production of a similar magnitude (~12 pmol/embryo/h) to that observed here (~10 pmol/oocyte/h, Figure 3). These data, in conjunction with high levels of lactate dehydrogenase in human embryos (Martin *et al.*, 1993), suggest that the lactate produced could be derived from pyruvate.

During the first incubation, pyruvate uptake was similar in the two media (Figure 2). However, during the second incubation, pyruvate uptake was 30% lower in MEME than in TCM 199. Levels of glutamine are >4-fold higher in MEME compared with TCM 199 (Table I). Glutamine is metabolized by both maturing cow and cat oocytes (Rieger and Loskutoff, 1994; Steeves and Gardner, 1999a; Spindler *et al.*, 2000). As both pyruvate and glutamine are tricarboxylic acid (TCA) cycle precursors, it can be hypothesized that the high levels of glutamine found in MEME may compete with pyruvate for entry into the TCA cycle, resulting in lower pyruvate uptake under these conditions. Alternatively, the presence of Tween 80 in TCM 199 could result in the oocyte membrane becoming more permeable with time, leading to higher pyruvate uptake during the second incubation.

The significant differences in pyruvate uptake between oocytes undergoing GVBD and those extruding their polar body when cultured in MEME are intriguing. Cyclic AMP

(cAMP) is involved in the maintenance of meiosis in a number of species (Downs, 1995a; review) and arises from ATP by the action of adenylate cyclase. During spontaneous maturation *in vitro*, cAMP levels decrease in oocytes (Downs, 1995a), and this is associated with a decrease in ATP levels (Downs, 1995b). It is possible that once the oocyte is irreversibly committed to mature, cAMP is no longer required to maintain meiotic arrest, ATP requirements fall and pyruvate uptake decreases. Interestingly, pyruvate uptake by oocytes which failed to mature remained high throughout the 24 h period.

Maturation of human oocytes *in vitro* is accompanied by changes in the aggregation patterns of mitochondria (Sathananthan and Trounson, 2000; Wilding *et al.*, 2001), which may reflect changing metabolic needs of the oocyte in preparation for fertilization. These changes in mitochondrial distribution may also account for the lower pyruvate uptake that was observed during later stages of nuclear maturation.

This work has shown for the first time that it is possible to measure pyruvate uptake and lactate production by individual, maturing human oocytes non-invasively. It also demonstrates that the metabolism of human oocytes can be significantly influenced by both the composition of the in-vitro culture medium and the cellular event taking place within the oocyte nucleus. The fact that the culture environment affects metabolism during human oocyte maturation suggests that the choice of culture medium for clinical IVM could be critical for embryonic and possibly fetal health. Further work is needed to investigate how substrate utilisation by maturing oocytes relates to subsequent development.

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