Changes in the metabolism of glucose, pyruvate, glutamine and glycine during maturation of cattle oocytes *in vitro*

D. Rieger and N. M. Loskutoff*

Animal Biotechnology Embryo Laboratory, Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

After maturation *in vitro* for 0, 6, 12, 18 or 24 h, the metabolism of radiolabelled glucose, glutamine, pyruvate and glycine by individual cattle oocytes was measured for 3 h. The metabolism of glucose through the Embden–Meyerhof (1.77–2.66 pmol per oocyte per 3 h) and pentose-phosphate (0.39–0.75 pmol per oocyte per 3 h) pathways was low and did not change over time. The oxidative metabolism of glucose carbon through the Krebs cycle was low throughout maturation, but increased significantly ($P \leq 0.05$) at 6 h (0.41 pmol per oocyte per 3 h) and 18 h (0.69 pmol per oocyte per 3 h). Pyruvate, glutamine and glycine metabolism in the Krebs cycle increased during culture. Pyruvate metabolism increased significantly from 0 h (17.3 pmol per oocyte per 3 h) to 6 h (23.3 pmol per oocyte per 3 h) and reached a maximum at 12 h (30.8 pmol per oocyte per 3 h). Glutamine metabolism was unchanged from 0 to 12 h (0.89 pmol per oocyte per 3 h), and then increased significantly at 18 h (2.25 pmol per oocyte per 3 h). Glycine metabolism increased significantly from 6 h (0.21 pmol per oocyte per 3 h) to 12 h (0.46 pmol per oocyte per 3 h) and reached a maximum at 18 h (0.68 pmol per oocyte per 3 h). The results suggest that oxidative metabolism increases, and is the major site of cellular energy production, during maturation of the cattle oocyte *in vitro*.

Introduction

Biggers *et al.* (1967) showed that pyruvate or oxaloacetate, but not glucose, lactate or phosphoenolpyruvate, can support the maturation of denuded mouse oocytes through meiosis to metaphase II. Denuded oocytes produce significantly greater amounts of $^{14}\text{CO}_2$ from [^{14}C]pyruvate than from [^{14}C]glucose: 57 and 28 times greater in mice and rabbits, respectively, (Brinster, 1969), 15–20 times greater in Rhesus monkeys (Brinster, 1971), and up to 10 times greater in cattle (Khurana and Niemann, 1992). These observations suggest that mitochondrial oxidative metabolism is much more important than anaerobic glucose metabolism for energy production in the mammalian oocyte.

Zuelke and Brackett (1992) cultured cattle cumulus–oocyte complexes for 24 h and then measured glucose metabolism by the intact complex and by denuded oocytes. Total glucose metabolism was significantly greater by cumulus–oocyte complexes matured in the presence rather than in the absence of LH, but was undetectable in the denuded oocytes. They subsequently showed that glutamine metabolism was greater in intact cumulus–oocyte complexes and in denuded oocytes following maturation in the presence of LH than in its absence

(Zuelke and Brackett, 1993). Khurana and Niemann (1992) reported that pyruvate metabolism was significantly greater in cattle oocytes denuded after 24 h maturation *in vitro* than in immature oocytes. However, with the exception of a preliminary report of some of the results presented here (Rieger and Loskutoff, 1993), there are, to our knowledge, no published studies of the changes in the metabolic activity of cattle oocytes over the course of maturation *in vitro*.

As in previous studies of early cattle embryos (Rieger and Guay, 1988; Tiffin *et al.*, 1991; Rieger *et al.*, 1992a, b), the production of $^3\text{H}_2\text{O}$ from D-[5- ^3H]glucose was used as a measure of glucose metabolism through the Embden–Meyerhof pathway (to phosphoenolpyruvate), and the production of $^{14}\text{CO}_2$ from D-[1- ^{14}C]glucose and D-[6- ^{14}C]glucose was used to determine the relative amounts of glucose metabolized through the pentose-phosphate pathway and the Krebs cycle. The production of $^{14}\text{CO}_2$ from [2- ^{14}C]pyruvate and the production of $^3\text{H}_2\text{O}$ from L-[3,4- ^3H (N)]glutamine were used to measure mitochondrial oxidative metabolism. The production of $^3\text{H}_2\text{O}$ from [2- ^3H]glycine was measured because glycine is found in high concentrations in bovine follicular fluid (Ménézo and Testart, 1975) and bovine (Moore and Bondioli, 1993) and ovine (Ménézo, 1973) oviductal secretions, and because of the possibility that glycine may serve as a substrate for energy production in the early mouse embryo (Hobbs and Kaye, 1985; Khatchadourian *et al.*, 1991).

*Present address: Henry Doorly Zoo, 3701 South 10 St., Omaha, NE 68107-2200, USA.

Received 30 April 1993.

Materials and Methods

Oocyte preparation

For each experiment, 200–250 oocytes were recovered from approximately 20 ovaries obtained from an abattoir, as described by Xu *et al.* (1992). The ovaries were from mature, Holstein cows, of unknown age and reproductive status. Within 2 h after slicing the first ovary, cumulus–oocyte complexes with more than three compact layers of granulosa cells were pooled and cultured in groups of 20 at 38°C under 5% CO₂ in air in 100 µl microdrops of TCM-199 (Earle's salts) medium (Gibco; Grand Island, NY), supplemented with 25 mmol Hepes l⁻¹ (Sigma Chemical Co., St Louis, MO), 1 mmol L-glutamine l⁻¹ (Sigma), 2.5 mmol sodium pyruvate l⁻¹ (Sigma), 60 U penicillin ml⁻¹ and 60 µg streptomycin ml⁻¹ (Gibco), and 10% (v/v) steer serum (Cocalico Biologicals, Reamstown, PA). The microdrops were overlaid with 1.0 ml silicone oil, previously sterilized by filtration (0.22 µm) and equilibrated with the culture medium.

After 0, 6, 12, 18 and 24 h of culture, 25–30 cumulus–oocyte complexes were removed from the microdrops and stripped of cumulus cells (denuded) by gentle pipetting through a series of glass micropipettes with decreasing inner diameters (200–80 µm). At each time, 10 oocytes were immediately fixed in methanol:acetic acid (3:1) as described by Xu *et al.* (1986). The other 15–20 oocytes were passed through four 1 ml washes of metabolic measurement medium, which was the same as the culture medium described above, but without serum. Of these, 8–10 oocytes were subjected to the 3 h metabolic measurement (see below), while the rest were cultured in the last wash for 3 h at 38°C under 5% CO₂ in air, as controls. The denuded control and measured oocytes were fixed after the 3 h metabolic measurement (i.e. at 3, 9, 15, 21 and 27 h). An additional 5–10 cumulus–oocyte complexes were removed from the culture drops at those times, stripped of cumulus cells and fixed immediately.

Forty-eight hours after fixation, the oocytes were stained with 1% aceto-orcein and evaluated for meiotic stage exactly as described by Xu *et al.* (1986). The oocytes were examined by phase contrast and Nomarski optics at magnification of × 250 to identify the stages of meiosis. The criteria used to define these specific stages were the same as those reported by Xu *et al.* (1986), except that germinal vesicle and germinal vesicle breakdown were used in the present study instead of oocyte nucleus stages I and II, respectively. Briefly, the criteria for the stages were as follows – germinal vesicle: there was a distinct nuclear envelope; germinal vesicle breakdown: the nuclear envelope still present but less distinct; diakinesis: the nuclear envelope was no longer visible but the chromatin remained within the area of the nucleus; late diakinesis: the nucleus area had totally disappeared; metaphase I: the chromosomes were maximally condensed; anaphase–telophase I: the chromosomes were under division or segregation; metaphase II: the contracted metaphase chromosomes and the first polar body were both present.

Metabolic measurements

Except for the medium, the technique used to measure oocyte metabolism was exactly as described for early cattle

embryos by Rieger *et al.* (1992b). All radiolabelled substrates were purchased from New England Nuclear (Mississauga, Ontario). The mixtures used for each experiment were: Expt 1: [2-¹⁴C]pyruvate (specific activity 10.4 mCi mmol⁻¹) with D-[5-³H]glucose (15.4 Ci mmol⁻¹); Expt 2: D-[1-¹⁴C]glucose (55.4 mCi mmol⁻¹) with L-[3,4-³H(N)]glutamine (30.0 Ci mmol⁻¹); and Expt 3: D-[6-¹⁴C]glucose (51.8 mCi mmol⁻¹) with [2-³H]glycine (48.4 Ci mmol⁻¹). Mixtures of one ¹⁴C-labelled and one ³H-labelled substrate were dried under nitrogen and taken up in metabolic measurement medium to give nominal concentrations of 0.05 µCi µl⁻¹ of [2-¹⁴C]pyruvate and 0.25 µCi µl⁻¹ for the other labelled substrates. Individual oocytes were taken up in 2 µl of the final wash of metabolic measurement medium and placed in the cap of a sterile 2.5 ml polypropylene screw-cap microtube together with 2 µl of radiolabelled substrate mixture (total culture volume 4 µl). The final substrate concentrations (labelled plus unlabelled) were 6.9 mmol pyruvate l⁻¹ and 5.55 mmol glucose l⁻¹ in Expt 1; 7.6 mmol glucose l⁻¹ and 1.7 mmol glutamine l⁻¹ in Expt 2; and 8.7 mmol glucose l⁻¹ and 0.6 mmol glycine l⁻¹ in Expt 3. The cap was quickly fitted onto the tube, which had been loaded with 1.5 ml of 25 mmol NaHCO₃ l⁻¹ equilibrated with a gas mixture of 5% O₂, 5% CO₂ and 90% N₂. Two or three sham preparations, containing all reagents but no embryo, were included for each mixture of labelled substrates in each measurement period. These served as control for all nonspecific radioactivity due to machine background, chemiluminescence, bacterial contamination and spontaneous breakdown of the labelled substrate. At the end of the 3 h measurement period, the caps were removed and the bicarbonate was mixed with 15 ml of scintillation fluid and radioactivity was measured for 10 min in a liquid scintillation counter. The total radioactivity (d.p.m.) of labelled substrate was determined by mixing 2 µl of the labelled substrate solution with 1.5 ml of 25 mmol NaHCO₃ l⁻¹, and measuring radioactivity in the same way. The amount of each substrate metabolized by each embryo was calculated as described by Tiffin *et al.* (1991). For each labelled substrate, the mean radioactivity (d.p.m.) for the sham preparations was subtracted from the radioactivity for each embryo. The difference was divided by the total radioactivity of labelled substrate added, and multiplied by the total quantity of substrate (labelled plus unlabelled) in 4 µl of medium, and by a product recovery correction factor of 1.27 or 1.41 for the ³H-labelled and ¹⁴C-labelled substrates, respectively (Rieger *et al.*, 1992b).

Statistical analysis

The proportion of oocytes at an identifiable stage after fixation, and the distributions of oocytes across meiotic stages after each measurement period for the immediately fixed, denuded controls and measured oocytes (combined across all experiments) were compared by χ^2 analysis. The effect of maturation time on the metabolism of each substrate in each experiment was evaluated by one-way analysis of variance of log(*y* + 1), where *y* = the measured metabolism. The log-transformed means were compared by Duncan's multiple-range tests.

Table 1. Meiotic stage of cattle oocytes matured *in vitro* and fixed at the indicated culture times

Culture time (h)	Group	Number of identified oocytes	Percentage of oocytes at the meiotic stage of					
			GV	GVBD	D	LD	MI	ATI
0	Cumulus enclosed	25	52.0	48.0				
3	Cumulus enclosed	19		89.5	10.5			
3	Denuded controls	11	9.1	90.9				
3	Denuded measured	25	32.0	52.0	16.0			
6	Cumulus enclosed	24	4.2	50.0	45.8			
9	Cumulus enclosed	16			100.0			
9	Denuded controls	11			100.0			
9	Denuded measured	26		15.4	76.9		7.7	
12	Cumulus enclosed	26		11.5	11.5	61.5	15.4	
15	Cumulus enclosed	19			10.5	31.6	31.6	26.3
15	Denuded controls	14		7.1		64.3	28.6	
15	Denuded measured	26	3.8		7.7	80.8	7.7	
18	Cumulus enclosed	24		4.2	4.2	12.5	8.3	
21	Cumulus enclosed	10	10.0			20.0		
21	Denuded controls	14						7.1
21	Denuded measured	24				8.3		4.2
24	Cumulus enclosed	20					10.0	
27	Cumulus enclosed	10						
27	Denuded controls	12						
27	Denuded measured	18					11.1	

Cumulus-enclosed oocytes were cultured within the cumulus cells until the time of fixation. Denuded, control oocytes were stripped of cumulus cells and cultured for 3 h before fixation. Denuded, measured oocytes were stripped of cumulus cells and their metabolism measured for 3 h before fixation. GV: germinal vesicle; GVBD: germinal vesicle breakdown; D: diakinesis; LD: late diakinesis; MI: metaphase I; ATI: anaphase–telophase I; MII: metaphase II.

Results

The cumulus oophorus expanded during *in vitro* maturation. From 12 h of culture onwards, the denuding procedure became more difficult because of increasing mucin production by the cumulus cells.

The meiotic stage was identified for 374 of 461 oocytes fixed (81.1%) after 0–27 h of culture (Table 1). The proportion of identified oocytes was not different ($P > 0.05$) among those fixed immediately after removing the cumulus cells (76.8%), after the 3 h metabolic measurement (85.0%), or after a 3 h control culture (88.7%). At the beginning of culture, the oocytes were at the germinal vesicle (52%) or germinal vesicle breakdown (48%) stage. All the cumulus-enclosed oocytes reached the diakinesis stage by 9 h, 57.9% reached metaphase I or anaphase–telophase I by 15 h and 100% reached metaphase II by 27 h. There were no significant differences in the distribution across meiotic stage among the cumulus-enclosed, denuded controls, and measured oocytes at 9, 21 or 27 h ($P > 0.10$). However, at 3 h, only 68% of the measured denuded oocytes reached the germinal vesicle breakdown or diakinesis stage, compared with 90.9% of the denuded controls, and 100% of the cumulus-enclosed oocytes ($P \leq 0.05$). At 15 h, only 7.7% of the measured oocytes had reached the metaphase I stage, compared with 28.6% of the denuded controls, and 57.9% for the cumulus-enclosed oocytes ($P \leq 0.05$).

Analysis of variance of the results of Expt 1 (Table 2) indicated a significant effect of maturation culture time on the metabolism of [2- 14 C]pyruvate ($P \leq 0.02$). The metabolism of

[2- 14 C]pyruvate increased significantly from 0 to 6 h ($P \leq 0.05$) and reached a maximum value at 12 h. The metabolism of [5- 3 H]glucose did not change significantly throughout maturation ($P > 0.50$).

In Expt 2 (Table 3), there was no significant change in the metabolism of [1- 14 C]glucose ($P > 0.15$). There was a very highly significant overall effect of maturation time on the metabolism of [3,4- 3 H]glutamine metabolism ($P \leq 0.001$), which was unchanged from 0 to 12 h, but increased significantly between 12 and 18 h.

There was a very highly significant effect of maturation time on the metabolism of [6- 14 C]glucose ($P \leq 0.0002$) and [2- 3 H]glycine ($P \leq 0.0001$, Expt 3, Table 4). The metabolism of [6- 14 C]glucose was undetectable at 0 h of culture, but increased at 6 h, decreased at 12 h, and then reached a maximum value at 18 h before falling to undetectable values at 24 h. The metabolism of [2- 3 H]glycine was unchanged from 0 to 6 h, increased at 12 h to reach a maximum at 18 h, and then decreased at 24 h of culture.

Discussion

The proportion of oocytes undergoing germinal vesicle breakdown at 0 h (48%) was higher in this study than the 25% previously reported by Xu *et al.* (1986). However, the oocytes examined at 0 h, as defined in this study, may have actually been liberated from the follicles for as long as 2 h before fixation. Mammalian oocytes spontaneously resume meiotic maturation once they are liberated from antral follicles

Table 2. Metabolism of [2-¹⁴C]pyruvate and [5-³H]glucose by individual, denuded, cattle oocytes over a 3 h period beginning at the indicated culture times

Culture time (h)	Number of oocytes	Labelled substrate	
		[2- ¹⁴ C]pyruvate (pmol per oocyte per 3 h)	[5- ³ H]glucose (pmol per oocyte per 3 h)
0	10	17.3 ± 3.04 ^a	2.07 ± 0.29
6	10	23.3 ± 1.22 ^b	2.16 ± 0.54
12	8	30.8 ± 3.76 ^b	2.00 ± 0.62
18	9	19.4 ± 1.98 ^{a,b}	1.77 ± 0.37
24	9	22.0 ± 1.75 ^{a,b}	2.26 ± 0.31

All values are means ± SEM.

^{a,b}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

Table 3. Metabolism of [1-¹⁴C]glucose and [3,4-³H]glutamine by individual, denuded, cattle oocytes over a 3 h period beginning at the indicated culture times

Culture time (h)	Number of oocytes	Labelled substrate	
		[1- ¹⁴ C]glucose (pmol per oocyte per 3 h)	[3,4- ³ H]glutamine (pmol per oocyte per 3 h)
0	8	0.74 ± 0.11	0.72 ± 0.11 ^a
6	9	0.39 ± 0.11	1.08 ± 0.15 ^a
12	9	0.75 ± 0.24	0.89 ± 0.16 ^a
18	10	0.40 ± 0.13	2.25 ± 0.28 ^b
24	10	0.74 ± 0.14	2.46 ± 0.65 ^b

All values are means ± SEM.

^{a,b}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

Table 4. Metabolism of [6-¹⁴C]glucose and [2-³H]glycine by individual, denuded, cattle oocytes over a 3 h period beginning at the indicated culture times

Culture time (h)	Number of oocytes	Labelled substrate	
		[6- ¹⁴ C]glucose (pmol per oocyte per 3 h)	[2- ³ H]glycine (pmol per oocyte per 3 h)
0	10	0 ± 0 ^a	0.15 ± 0.08 ^a
6	9	0.41 ± 0.12 ^{b,c}	0.21 ± 0.07 ^a
12	10	0.18 ± 0.13 ^{a,b}	0.46 ± 0.09 ^{b,c}
18	9	0.69 ± 0.21 ^c	0.68 ± 0.07 ^c
24	10	0 ± 0 ^a	0.42 ± 0.08 ^b

All values are means ± SEM.

^{a,b,c}Within columns, values with different superscripts are significantly different ($P \leq 0.05$).

(Edwards, 1965; Schroeder and Eppig, 1984). The oocytes examined in this study also matured at a faster rate than those reported by Xu *et al.* (1986); 71% versus 25% of the oocytes reached metaphase II after 18 h of *in vitro* culture, respectively. The reasons for these differences are unclear, but may be due to differences in the concentrations of LH (Dominko and First,

1992) or other factors present in the culture media used for *in vitro* maturation; the medium Xu *et al.* (1986) used contained 20% fetal calf serum, whereas 10% steer serum was used in the present study.

A proportion of the oocytes subjected to the metabolic measurement procedure matured during the measurement

period, at all tested times. The maturation of oocytes fixed at 9, 21 and 27 h did not differ among the measured, denuded controls and cumulus enclosed groups, but maturation was retarded in the measured oocytes fixed at 3 and 15 h. This finding suggests that the cellular processes involved in germinal vesicle breakdown and late diakinesis are especially dependent on the cumulus cells, or are more sensitive to the mildly adverse conditions associated with transfer of the oocytes to the metabolic measurement apparatus. We have previously observed that cattle embryos at the early cleavage stage are more sensitive to the metabolic measurement procedure than are embryos at later stages (Rieger *et al.*, 1992a), possibly owing to the very short-term exposure to ambient temperature (Loskutoff *et al.*, 1993).

Total glucose metabolism (as measured by the production of $^3\text{H}_2\text{O}$ from $[5\text{-}^3\text{H}]\text{glucose}$) and the production of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{glucose}$ were low and relatively constant throughout maturation. Khurana and Niemann (1992) observed that production of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]\text{glucose}$ increased during maturation of cattle oocytes, but never exceeded 1 pmol per embryo per h. We observed very similar values for the metabolism of these labelled substrates by two- to eight-cell cattle embryos, which led us to conclude that glucose metabolism is limited at these early stages before the activation of the embryonic genome (Rieger *et al.*, 1992a).

In contrast to the relatively constant metabolism of $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[5\text{-}^3\text{H}]\text{glucose}$, the production of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{glucose}$ varied markedly during maturation. At 0 and 24 h, there was no measurable production of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{glucose}$ and, therefore, the production of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{glucose}$ (0.74 pmol per oocyte per 3 h) can be considered to have arisen exclusively from processing through the pentose-phosphate pathway (see Larrabee, 1989). It is unclear why glucose is metabolized through the Embden–Meyerhof pathway as far as phosphoenolpyruvate, but glucose carbon is not metabolized through the Krebs cycle. This might result from inhibition of pyruvate kinase, as we have suggested occurs in cattle blastocysts (Rieger and Guay, 1988), or that glucose is converted to lactate and secreted from the oocyte, as has been observed in early cleavage-stage rat and human embryos (see Leese *et al.*, 1993).

The significant increases in production of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{glucose}$ at 6 and 18 h, when there were no corresponding increases in production of $^{14}\text{CO}_2$ from $[1\text{-}^{14}\text{C}]\text{glucose}$, suggest that the production of $^{14}\text{CO}_2$ from $[6\text{-}^{14}\text{C}]\text{glucose}$ at those times arose from oxidative metabolism in the Krebs cycle. At 18 h, this apparent mitochondrial metabolism represented 39% of total glucose metabolism.

Increasing oxidative metabolism is also indicated by the significant increases in the metabolism of $[2\text{-}^{14}\text{C}]\text{pyruvate}$ and $[3,4\text{-}^3\text{H}]\text{glutamine}$ during maturation, reaching their maxima at 12 and 18–24 h, respectively. Khurana and Niemann (1992) also observed a significant increase in the metabolism of $[2\text{-}^{14}\text{C}]\text{pyruvate}$ following maturation *in vitro*, although their measurements were lower by a factor of ten. The maximum metabolism of pyruvate by cattle oocytes in the present study was four times greater, and that of glutamine, three times less, than our previous measurements in two-cell cattle embryos (Rieger *et al.*, 1992a, b). However, it is unclear whether these differences reflect metabolic changes between the oocyte and

two-cell stage, or are due to differences in the media used for the metabolic measurements.

The results suggest that glycine can also serve as an energy substrate for cattle oocytes. The production of $^3\text{H}_2\text{O}$ from $[2\text{-}^3\text{H}]\text{glycine}$ involves the formation of serine and then pyruvate as intermediates (Voet and Voet, 1990). It is, therefore, not surprising that the pattern of change in glycine metabolism during maturation was similar to that of pyruvate, increasing from 0 h to a maximum at 18 h.

The patterns of metabolic activity during maturation of cattle oocytes in the present study are consistent with changes in the enzyme activities of rat oocytes during maturation *in vivo*, as reported by Tsutsumi *et al.* (1992). In their study, the activities of hexokinase and phosphofructokinase, enzymes of the Embden–Meyerhof pathway, increased during maturation while the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, enzymes of the pentose-phosphate pathway, decreased. However, the limited metabolism of $[5\text{-}^3\text{H}]\text{glucose}$ and $[1\text{-}^{14}\text{C}]\text{glucose}$ by cattle oocytes in the present study is consistent with the observation that hexokinase activity is very low throughout maturation of rat oocytes (Tsutsumi *et al.*, 1992), and, therefore, rate-limiting for both the Embden–Meyerhof and pentose-phosphate pathways. The increases in the metabolism of $[2\text{-}^{14}\text{C}]\text{pyruvate}$, $[3,4\text{-}^3\text{H}]\text{glutamine}$, $[6\text{-}^{14}\text{C}]\text{glucose}$, and $[2\text{-}^3\text{H}]\text{glycine}$ by cattle oocytes are consistent with the observations that the activity of malate dehydrogenase, an enzyme of the Krebs cycle, doubles (Tsutsumi *et al.*, 1992), and oxygen consumption increases (Magnusson *et al.*, 1977), during maturation of rat oocytes.

The changes in oxidative metabolism may also be related to ultrastructural changes of cattle oocytes during maturation *in vitro*. Hyttel *et al.* (1986) and Shamsuddin *et al.* (1993) reported that mitochondria move from the periphery of cattle oocytes, to become distributed throughout the ooplasm between 12 and 20 h of maturation *in vitro*, approximately coincident with the increases in the metabolism of Krebs cycle substrates in the present study. However, further investigation will be necessary to determine whether there is a direct physiological relationship between these observations.

In the present study, the metabolic activity of cattle oocytes was measured after removal of the cumulus cells. These conditions are quite different from the natural situation of metabolic coupling in which glycolytic intermediates, amino acids, ATP and other small molecules produced by the cumulus cells may be secreted and taken up by the oocyte, or transferred into the oocyte through gap junctions (see Eppig, 1991). Pyruvate or oxaloacetate alone can support the maturation of denuded mouse oocytes, but glucose can do so only when follicular cells are present in the culture medium (Biggers *et al.*, 1967). This finding suggests that the cumulus cells metabolize glucose to pyruvate or Krebs cycle intermediates which are passed to the oocyte. To our knowledge, the concentrations of energy substrates to which the cumulus-enclosed oocyte is exposed are completely unknown and may be very different from the concentrations in the medium in this study. Consequently, the measurements of metabolism by denuded cattle oocytes, like all other studies of denuded oocytes, may not necessarily correspond to the absolute activities of the relevant metabolic pathways in

cumulus-enclosed oocytes. However, these measurements do provide an indication of the potential of the oocyte to metabolize the tested substrates, and the patterns of change in their metabolism probably reflect similar changes in the cumulus-enclosed oocyte.

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada, the Ontario Ministry of Agriculture and Food, the Premier's Council Technology Fund of Ontario, and Semex Canada. The authors thank L. Afflu, N. Rumph and E. Semple for collection and preparation of the oocytes, and J. W. Pollard for his critique of the manuscript.

References

- Biggers JD, Whittingham DG and Donahue RP (1967) The pattern of energy metabolism in the mouse oocyte and zygote *Proceedings of the National Academy of Science* **58** 560–567
- Brinster RL (1969) Radioactive carbon dioxide production from pyruvate and lactate by the preimplantation rabbit embryo *Experimental Cell Research* **54** 205–209
- Brinster RL (1971) Oxidation of pyruvate and glucose by oocytes of the mouse and rhesus monkey *Journal of Reproduction and Fertility* **24** 187–191
- Dominko T and First NL (1992) Kinetics of bovine oocyte maturation allows selection for developmental competence and is affected by gonadotropins *Theriogenology* **37** 203 (Abstract)
- Edwards RG (1965) Maturation *in vitro* of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes *Nature* **208** 349–351
- Eppig JJ (1991) Intercommunication between mammalian oocytes and companion somatic cells *Bioessays* **13** 569–574
- Hobbs JG and Kaye PL (1985) Glycine transport in mouse eggs and preimplantation embryos *Journal of Reproduction and Fertility* **74** 77–86
- Hyttel P, Xu KP, Smith S and Greve T (1986) Ultrastructure of *in-vitro* oocyte maturation in cattle *Journal of Reproduction and Fertility* **78** 615–625
- Khatchadourian C, Guillaud J and Ménézo Y (1991) Importance of glycine for the mouse one cell zygote and blastocyst metabolism: conversion and absence of a glycine methyl transferase activity *Experimental and Clinical Endocrinology* **10** 45–51
- Khurana NK and Niemann H (1992) Energy metabolism of bovine oocytes matured and fertilized *in vitro* *Proceedings of the 12th International Congress on Animal Reproduction, The Hague, Vol. 1*, 348–350 (Abstract)
- Larrabee M (1989) The pentose cycle (hexose monophosphate shunt). Rigorous evaluation of limits to the flux from glucose using $^{14}\text{CO}_2$ data, with applications to peripheral ganglia of chicken embryos *Journal of Biological Chemistry* **264** 15875–15879
- Leese HJ, Conaghan J, Martin KL and Hardy K (1993) Early human embryo metabolism *Bioessays* **15**, 259–264
- Loskutoff NM, Johnson WH and Betteridge KJ (1993) The developmental competence of bovine embryos with reduced cell numbers *Theriogenology* **39** 95–107
- Magnusson C, Hillensjo T, Tsafri A, Hultborn R and Ahren K (1977) Oxygen consumption of maturing rat oocytes *Biology of Reproduction* **17** 9–15
- Ménézo YJR (1973) Amino constituents of tubal and uterine fluids of the estrous ewe: comparison with blood serum and ram seminal fluid. In *The Biology of Spermatozoa*, pp 174–181 Eds ESE Hafez and CG Thibault. S. Karger AG Basel
- Ménézo Y and Testart J (1975) Etude comparée du sérum sanguin et du liquide folliculaire préovulatoire chez la vache *Annales de Biologie Animale, Biochimie, Biophysique* **15** 1–8
- Moore K and Bondioli KR (1993) Glycine and alanine supplementation of culture medium enhances development of *in vitro* matured and fertilized cattle embryos *Biology of Reproduction* **48** 833–840
- Rieger D and Guay P (1988) Measurement of the metabolism of energy substrates in individual bovine blastocysts *Journal of Reproduction and Fertility* **83** 585–591
- Rieger D and Loskutoff NM (1993) Changes in the metabolism of glucose, pyruvate and glutamine during *in-vitro* maturation of bovine oocytes *Theriogenology* **39** 298 (Abstract)
- Rieger D, Loskutoff NM and Betteridge KJ (1992a) Developmentally related changes in the metabolism of glucose and glutamine by cattle embryos produced and co-cultured *in vitro* *Journal of Reproduction and Fertility* **95** 585–595
- Rieger D, Loskutoff NM and Betteridge KJ (1992b) Developmentally related changes in the uptake and metabolism of glucose, glutamine and pyruvate by cattle embryos produced *in vitro* *Reproduction Fertility and Development* **4** 547–557
- Schroeder AC and Eppig JJ (1984) The developmental capacity of mouse oocytes that matured spontaneously *in vitro* is normal *Developmental Biology* **102** 493–497
- Shamsuddin M, Larsson B and Rodriguez-Martinez H (1993) Maturation-related changes in bovine oocytes under different culture conditions *Animal Reproduction Science* **31** 49–60
- Tiffin G, Rieger D, Betteridge KJ, Yadav BR and King WA (1991) Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development *Journal of Reproduction and Fertility* **93** 125–132
- Tsutsumi O, Satoh K, Taketani Y and Kato T (1992) Determination of enzyme activities of energy metabolism in the maturing rat oocyte *Molecular Reproduction and Development* **33** 333–337
- Voet D and Voet JG (1990) *Biochemistry*, p 1223. John Wiley and Sons, Inc., New York
- Xu KP, Greve T, Smith S and Hyttel P (1986) Chronological changes of bovine follicular oocyte maturation *in vitro* *Acta veterinaria Scandinavica* **27**, 505–519
- Xu KP, Yadav BR, Rorie RW, Plante L, Betteridge KJ and King WA (1992) Development and viability of bovine embryos derived from oocytes matured and fertilized *in vitro* and co-cultured with bovine oviducal epithelial cells *Journal of Reproduction and Fertility* **94** 33–43
- Zuelke KA and Brackett BG (1992) Effects of luteinizing hormone on glucose metabolism in cumulus-enclosed bovine oocytes matured *in vitro* *Endocrinology* **131** 2690–2696
- Zuelke KA and Brackett BG (1993) Increased glutamine metabolism in bovine cumulus-cell enclosed and denuded oocytes after *in vitro* maturation with luteinizing hormone *Biology of Reproduction* **48** 815–820