In-vitro uptake of glucose by bovine blastocysts

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Summary. Blastocysts, obtained from cows on Day 10–11 after oestrus, were cultured for 20 h. Most (81·3%) blastocysts grew in culture and about 50% took up glucose. There was no morphological difference between the blastocysts which did or did not take up glucose but development *in vivo* was better for blastocysts which had taken up glucose (69·2%) than for those which did not (14·2%).

Introduction

The prediction of the viability of bovine embryos is an important factor in improving the pregnancy rate after transfer. At present, morphological criteria are used to classify such embryos but this method of assessment is subjective and dependent on wide previous experience (Shea, Hines, Lightfoot, Ollis & Olson, 1976; Trounson, Willadsen, Rowson & Newcomb, 1976; Elsdén, Nelson & Seidel, 1978). The ability of embryos to hatch from the zona pellucida provides a simple *in-vitro* check of the viability of cultured embryos (Renard & du Mesnil du Buisson, 1976). Furthermore, glucose is essential for the hatching process; zona loss is completely inhibited when glucose is replaced by pyruvate and lactate (J.-P. Renard, unpublished observations). These findings led us to study the possibility of using glucose uptake *in vitro* as a biochemical indicator of the viability of bovine embryos before transfer.

Materials and Methods

Blastocysts were recovered on Day 10–11 (Day 0 = day of oestrus) from Charolais heifers induced to superovulate by a single injection of 2100 i.u. PMSG (Folligon: Intervet) followed 48 h later by an intramuscular injection of 750 µg prostaglandin analogue (ICI 80,996). Donors were inseminated twice with frozen bull semen 56 and 72 h after prostaglandin injection. The 59 hatched blastocysts used for the experiment were collected by a cervical technique described elsewhere (Ozil, Heyman & Renard, 1979), and placed in B2 medium (Menezo, 1976) for morphological examination. Within 30 min of collection, each embryo was placed in a sterile glass tube (9 × 75 mm) in 0·5 ml B2 medium containing 600 µg glucose, gassed with 90% N₂, 5% O₂, 5% CO₂ and cultured at 37°C for 20 ± 2 h.

The size of the embryo and the concentration of glucose in the medium were measured before and after culture. Size was measured with an ocular micrometer and was defined as the diameter for spherical embryos and the square root of the product of length and width for elongated embryos. Histological techniques were used to determine the number of cells in some embryos after culture. Glucose uptake was estimated by the hexokinase method (Glucoquant: Boehringer) in the medium before and after culture. When the difference in glucose concentration before and after culture was 50 µg/0·5 ml or less (uptake <2·5 µg/embryo/h), it was considered that there was no glucose uptake by the embryo. This figure was derived from determinations of the repeatability of the assay using B2 medium without embryo (control).

The viability of 27 embryos which increased in size during culture was examined *in vivo* by single cervical transfer, using the technique described by Renard, Heyman & du Mesnil du Buisson (1977). The onset of oestrus in the recipients was exactly synchronized with that of the donors by the injection of 500 µg prostaglandin analogue. After transfer, the absence of return to oestrus and increased plasma progesterone concentrations were used for early determination of pregnancy. The presumed pregnant recipients were slaughtered between Days 45 and 52 of pregnancy for the observation of the fetuses.

Significant differences and homogeneity between samples were determined by statistical analysis using a *t* test for differences of paired measurements and a χ^2 test with Yates' correction for proportions. We computed the correlation coefficient between the sizes of blastocysts before and after culture.

Results

After 20 h in culture, 48 out of 59 embryos (81.3%) had increased in size (mean rate of increase 56%). From these 48 embryos, only 25 (52.1%) showed a significant uptake of glucose from the culture medium. The mean amount of glucose taken up by these embryos (Table 1) was 5.81 ± 0.70 µg/h. Considerable variability was recorded in uptake rates of embryos of the same size, but the proportions of embryos taking up glucose did not differ significantly with the final size class. The initial and final mean sizes of embryos that enlarged in culture and took up glucose did not differ from those of the 23 embryos that enlarged in culture but showed no glucose uptake (Table 2). High correlations were found between sizes before and after culture in both groups of embryos.

Table 1. Mean $\pm$ s.e.m. amount of glucose taken up by bovine blastocysts that enlarged in culture

	Final size of embryo after culture (µm)			
	150–300	300–450	450–600	600–750
No. of embryos cultured	13	16	13	6
No. taking up glucose	7	9	6	3
Glucose uptake (µg/h)	4.36 ± 1.06	5.87 ± 0.92	7.41 ± 2.07	7.44 ± 2.06

Table 2. Initial and final mean ($\pm$ s.e.m.) sizes of bovine blastocysts in relation to glucose uptake

	Embryos with glucose uptake (N = 25)	Embryos with no glucose uptake (N = 23)	Total (N = 48)
Initial size (µm)	312.6 ± 43.1	263.1 ± 28.6	288.9 ± 26.3
Final size (µm)	$*472.6 \pm 63.6$	$*429.5 \pm 37.5$	$*452 \pm 37.5$
Increase	160	166	163.1
(rate)	(51.2%)	(63.1%)	(56.4%)
Correlation (<i>r</i>) between initial and final sizes	0.962	0.833	0.922

* $P < 0.05$ compared with initial size.

The counts of the number of cells in 14 embryos that had increased in size during culture gave similar values for embryos with (534 ± 86 cells, $N = 7$) or without (538 ± 86 cells, $N = 7$) glucose uptake. It was not possible to see any morphological differences between the 2 groups of embryos that enlarged in culture. Of the 59 embryos used, 11 (18.7%) failed to enlarge in culture. The mean ($\pm$ s.e.m.) initial size of these embryos was 305.3 ± 47.6 µm. Only 2 embryos

(initial sizes, 162 and 678 μm) took up glucose from the medium (3.15 and 10.3 $\mu\text{g/h}$ respectively); the 9 other embryos showed no glucose uptake.

The development of embryos *in vivo* after cervical transfer is shown in Table 3. Significantly more embryos which exhibited uptake developed than did those with no glucose uptake.

Table 3. Development of cultured bovine embryos after single cervical transfer

	Embryos showing glucose uptake	Embryos with no glucose uptake	Total
No. of recipients	13	14	27
No. of pregnancies at Day 50	9*	2*	11
(%)	(69.2)†	(14.2)	(40.7)

* Significantly different ($\chi^2 = 6.30$; $P < 0.025$).

† Including 1 dead fetus.

Discussion

When Day 10–11 (hatched) blastocysts were cultured for 20 h, 80% of them increased in size. The final mean size was not significantly different from that of freshly collected Day-12 embryos ($455 \pm 41.4 \mu\text{m}$, $N = 20$, unpublished data). A previous study showed that Day-10 embryos with a mean rate of increase in size of more than 50% during 24 h of culture had a significantly higher mean number of cells (+27%) than did freshly collected Day-10 blastocysts (Renard, Menez, Saumande & Heyman, 1978) and therefore increase in size does reflect some degree of development of the embryos during culture. However, the pregnancy rate achieved at Day 50 after transfer of individual embryos developed *in vitro* (40.7%) was lower than that obtained by direct transfer of uncultured embryos for which pregnancy rates of 50% resulted from surgical (Betteridge, Mitchell, Eaglesome & Randall, 1976) and cervical (Renard *et al.*, 1977) transfers, although the figure for transferred blastocysts which took up glucose was higher (69.2%).

Blastocysts cultured for 20 h develop more slowly *in vitro* than *in vivo*, the cell number of cultured Day-10 embryos being lower than that of uncultured embryos of the same size (Renard *et al.*, 1978), suggesting that optimal culture conditions have not yet been achieved. Groups of embryos which did not differ morphologically either before or after culture nevertheless exhibited different metabolic activity as demonstrated by glucose uptake in culture, corresponding to different potentialities for development after transfer. The low percentage of embryos that showed glucose uptake (46.9%) is presumably related to the culture conditions, as well as to the percentage of viable embryos at recovery.

In the mouse, glucose is a necessary factor *in vitro* for the hatching of blastocysts (Wordinger & Brinster, 1976) and this requirement may be related to a need for synthesis of glycogen (Ozias & Stern, 1973; Pike & Wales, 1973) and lipids (Flynn & Hillman, 1978). In the rabbit, glucose metabolism increases greatly during the period of rapid expansion of the blastocyst (Brinster, 1968). Assays carried out on Day-13 cow blastocysts revealed that 85% (12/14) had significant glucose uptake (unpublished data) with a wide range of variability (4.7–50 $\mu\text{g/h/embryo}$). These observations suggest that more accurate assessment could be obtained by using slightly older embryos; glucose uptake by Day-12–13 embryos cultured for periods of less than 20 h will be studied.

We thank Viviane Garnier and Marie-Claire Theron for skilful technical assistance and Aline Solari for performing the statistical analysis of the data. We are grateful to Dr R. M. Moor for reading and criticizing the manuscript.

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Received 25 April 1979