

Effects of taurine on human embryo development *in vitro* FREE

F. Devreker, M. Van den Bergh, J. Biramane, RM.L. Winston, Y. Englert, K. Hardy

Human Reproduction, Volume 14, Issue 9, September 1999, Pages 2350–2356, <https://doi.org/10.1093/humrep/14.9.2350>

Published: 01 September 1999 **Article history** ▼

Abstract

Glutamine and taurine are reported to be beneficial for mouse embryo development *in vitro*, and we have recently shown that glutamine improves human blastocyst formation *in vitro*. This randomized study compared the development of supernumerary human embryos in the presence of 1 mmol/l glutamine and/or 5 mmol/l taurine from the 2–4-cell stage to the blastocyst stage. Blastocyst development and cell numbers were similar in the presence of glutamine or taurine: 52.6% and 58.3% of the embryos reached the blastocyst stage, respectively. Pyruvate uptake was similar in the presence of glutamine or taurine throughout development, as was lactate production after the 8-cell stage. Before this stage, lactate production was 4-fold higher in the presence of taurine ($P < 0.001$). The proportion of embryos reaching the blastocyst stage was similar with glutamine alone or with glutamine and taurine (62.5% and 47.2% respectively), as were the blastocyst cell numbers (63.0 ± 4.6 and 61.0 ± 5.1 respectively). In conclusion, taurine supports development of 2–4-cell human embryos to the blastocyst stage, although it does not further augment the beneficial effects of glutamine.

Keywords: [embryo metabolism](#), [glutamine](#), [human blastocyst](#), [taurine](#)

Topic: [embryo stage 3](#), [cell count](#), [embryo](#), [taurine](#), [glutamine](#), [mice](#), [embryologic development](#)

Issue Section: [FERTILIZATION AND EARLY EMBRYOLOGY](#)

Introduction

Despite improvements in IVF, implantation rates remain disappointingly low. Only ~20% of transferred embryos implant ([Dawson et al., 1995](#)). In addition, ~50% of normally fertilized embryos cultured *in vitro* arrest during preimplantation development ([Hardy et al., 1993](#)). Embryo viability is partially compromised by suboptimal culture conditions although chromosomal abnormalities ([Kola et al., 1993](#)) or failure to activate the embryonic genome are other possible factors ([Tesarik, 1994](#)).

Recently, studies have been directed towards understanding the specific nutritional needs of embryos during development *in vitro*. Female reproductive tract fluids contain high concentrations of certain amino acids such as taurine, glutamine, glutamate or glycine ([Leese et al., 1979](#); [Borland et al., 1980](#); [Casslèn 1987](#); [Miller and Schultz 1987](#)) suggesting that they may play a role in preimplantation embryo development. Indeed, amino acids have been shown to maintain cellular functions, and promote both embryo development and differentiation ([Bavister, 1995](#)). Supplementation of culture media with amino acids is beneficial for the development *in vitro* of embryos from different species including mice ([Gardner and Lane, 1993](#)), hamster ([Bavister and Arlotto, 1990](#); [Bavister and McKiernan, 1993](#)), sheep ([Walker et al., 1996](#)), rabbit ([Kane and Foote, 1970](#)), rat ([Kishi et al., 1991](#)) and human ([Gardner et al., 1997](#)). Furthermore, amino acid supplementation promotes postimplantation development of mouse embryos when transferred to pseudopregnant recipients ([Lane and Gardner, 1994](#)). However, several amino acids such as proline, cysteine, arginine and phenylalanine have been shown to inhibit the development of hamster embryos ([Bavister and McKiernan, 1993](#)). Amino acids provide a pool of protein precursors, can regulate embryo metabolism and can act as an osmolyte or buffer the intracellular pH ([Gardner and Lane, 1997](#)). Medium supplemented with glutamine and non-essential amino acids for cleavage stage development, and supplemented with all

amino acids for blastocyst formation, provides the best support for mouse embryo development *in vitro*, with blastocysts having a potential almost equivalent to those developed *in vivo* (Lane and Gardner, 1997). Few studies have addressed the effects of specific amino acids on human embryo development *in vitro*.

Two amino acids in particular, glutamine and taurine, have been of interest in mammalian preimplantation embryo culture. Glutamine stimulated hamster embryo development and was found to be essential in the transition from the 8-cell stage to the morula stage (Carney and Bavister, 1987). It has been demonstrated that a glucose-free medium containing glutamine and EDTA alleviates the 2-cell block exhibited by embryos from outbred strains of mice (Chatot *et al.*, 1989). Glutamine also improves blastocyst formation from inbred and outbred strains of mice (Chatot *et al.*, 1990; Nasr-Esfahani *et al.*, 1992). Recently glutamine was found to enhance human embryo development *in vitro* (Devreker *et al.*, 1998).

Taurine is a β -amino acid present in high concentrations in many mammalian tissues, especially in the brain and in the heart (Huxtable, 1987). It is present in oviductal and uterine fluids from different species such as rabbit (Leese *et al.*, 1979) and mouse (Dumoulin *et al.*, 1992b). It is found in high concentrations in human uterine fluid, the concentration being higher at midcycle and during the luteal phase (Casslèn, 1987). Taurine is also present in human semen (Hervann *et al.*, 1986), the human sperm acrosome (Velazquez *et al.*, 1986) and in mouse and rabbit eggs (Schultz, 1981; Miller *et al.*, 1987). Taurine maintains sperm motility in hamster and human (Mrsny *et al.*, 1979) and is necessary for optimal fertilization of hamster oocytes (Gwatkin and Haidi, 1973). Taurine was found to promote embryo development from different species including mouse (Dumoulin *et al.*, 1992a, 1992b), pig (Reed *et al.*, 1992), cow (Liu and Foote, 1995), rabbit (Li *et al.*, 1993), and hamster (Bavister and McKiernan, 1993). However, little is known about the effect of taurine on human preimplantation embryo development *in vitro*.

Here we have investigated the effect of taurine on human preimplantation embryo development *in vitro* in the presence or absence of glutamine. The number of embryos that reached the

blastocyst stage and cell numbers in the trophectoderm and inner cell mass (ICM) were analysed.

Materials and methods

Source of human embryos

The study was initiated at Hammersmith Hospital in London and continued at Erasme Hospital in Brussels where the IVF clinic has an embryo freezing programme. Women were treated with human menopausal gonadotrophin (HMG; Pergonal[®], Serono, Welwyn Garden City, UK; Humegon[®], Organon, Oss, The Netherlands) in combination with luteinizing hormone-releasing hormone agonist (Buserelin[®]; Hoechst, Hounslow, UK). Ovulation was induced by injection of 10 000 IU of human chorionic gonadotrophin (HCG; Profasi[®], Serono; Pregnyl[®], Organon) and oocyte retrieval took place 34–36 h later.

Preincubation and insemination of oocytes, and embryo culture before transfer, were carried out in Earle's balanced salt solution (EBSS) containing 5.56 mmol/l glucose and supplemented with 25 mmol/l sodium bicarbonate (BDH, Lutterworth, UK; Sigma, Bornem, Belgium) and 0.47 mmol/l pyruvic acid, (Sigma) under a gas phase of 5% CO₂, 5% O₂ and 90% N₂ (Hillier *et al.*, 1984; Devreker *et al.*, 1996) in both laboratories. Media were supplemented with 10% maternal serum at Hammersmith Hospital or 0.5% human serum albumin at Erasme Hospital (Red Cross, Belgium). Four to 6 h after retrieval, individual oocyte–cumulus complexes were inseminated (day 0). Normal fertilization was confirmed 16h after insemination by the presence of two pronuclei (day 1). On the morning of embryo transfer (on day 2), embryos were examined and the number of cells determined. Each embryo was graded according to evenness of blastomeres, fragmentation and presence of cellular debris from perfectly symmetrical embryos with no fragmentation (grade I), through embryos with uneven blastomeres and/or presence of minor fragmentation (grade II), or with one blastomere fragmented (grade III), to embryos having one intact blastomere with gross fragmentation (grade IV) or being totally degenerate (grade V)

(Dawson *et al.*, 1995). A maximum of three embryos with the best morphology and at the most advanced stage of development were selected for transfer. At Erasme Hospital, remaining embryos of grade I or II were selected for the freezing programme.

The work was approved by research ethics committees at both units: the Royal Postgraduate Medical School, Hammersmith Hospital and licensed by the Human Fertilisation and Embryology Authority in the United Kingdom and the Free University of Brussels, Erasme Hospital in Belgium.

Preparation of culture media

Earle's balanced salt solution was prepared as described (Devreker *et al.*, 1998) without glucose. Media were prepared weekly from individual stock solutions and stored at 4 °C. All media were supplemented with 0.5% human serum albumin (HSA; Zenalb™, BPL, UK or Red Cross, Belgium) instead of maternal serum. Dishes were set up each day and equilibrated for at least 2 h in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂ at 37 °C before each experiment. Preparation of culture media, culture and scoring of embryos were performed by the same operator throughout the study.

Culture of embryos

The concentration of taurine was chosen according to mouse studies (Dumoulin *et al.*, 1992a,b) and the concentration in human genital tract fluids (Casslèn, 1987).

In experiment 1, the effect of taurine was examined in glucose-free medium. Following informed consent from 13 patients, untransferred normally fertilized embryos from each patient were evenly allocated to two treatment groups following block randomization. Embryos were cultured in glucose-free EBSS supplemented with 5 mmol/l taurine (Sigma) or 1 mmol/l L-glutamine (Sigma). Osmolarity of each batch of culture medium was measured using an osmometer (3 OM; Advanced Instrument Inc., Needham Heights, MA, USA) before embryo culture. The range of values was 280–287 mOsm. The pH was

measured using a Consort pH meter (Analis, Ghent, Belgium). The range of values was 7.5–7.6.

In experiment 2, the effect of taurine was examined in glucose-free medium in the presence of glutamine. Normally fertilized embryos, from 23 patients, unsuitable for transfer or freezing were allocated to two media following block randomization. Embryos were cultured in glucose-free EBSS containing 1 mmol/l glutamine with or without 5 mmol/l taurine.

For each experiment, the distributions of embryos of grade II or III were not different between the two groups, $P = 0.336$ (Mann–Whitney) for the first experiment and $P = 0.766$ (Mann–Whitney) for the second experiment (Figure 1). No embryos of grade I were available for these studies. However, the quality of the embryos included in the second experiment was significantly lower compared to the embryos included in the first experiment, $P = 0.001$ (Mann–Whitney). The implantation rates observed for the sibling transferred embryos were 10.7% and 21.4% for the first and the second experiments respectively. In the second experiment, 11 out of 23 patients had embryos suitable for freezing (Figure 1).

Embryos were individually incubated for sequential 24 h incubation periods between days 2 and 6 post-insemination in 5 μ l drops of medium overlaid with silicone fluid (Dow Corning 200/50 cs, BDH) (Hardy *et al.*, 1995). Similar drops of medium alone incubated adjacent to the embryo-containing drops served as controls. At the end of the culture period, each embryo was moved to a fresh incubation drop for the next 24 h. Individual 2 μ l aliquots of the spent and control drops of medium from the recently terminated culture were diluted with 398 μ l of a 5 μ mol/l solution of lactate (lactate standard; Sigma) in water.

The concentration of pyruvate and lactate in the control and incubation drops was determined by the method described by Hardy *et al.* (1995) based on a technique developed for non-invasive assessment of substrate uptake by mouse and human embryos (Leese *et al.*, 1984; Hardy *et al.*, 1989b). All these assays are based on the fluorescence of the coenzyme NADH when oxidized. The depletion of pyruvate and production of lactate by

the embryo was calculated by analysing the difference between substrate concentrations in the control and incubation drops. On day 6, the numbers of blastocysts were assessed and the proportion of cells in the trophectoderm and ICM was calculated.

Differential labelling of ICM and trophectoderm nuclei

The number of cells in the trophectoderm and ICM of expanded blastocysts were counted on the morning of day 6 as described previously ([Hardy *et al.*, 1989a](#)). TE nuclei were specifically labelled with the fluorochrome propidium iodide during antibody-mediated complement lysis. This fluorochrome is excluded from viable ICM cells. The whole embryo was then rapidly fixed in absolute ethanol and both TE and ICM nuclei labelled with a second fluorochrome, bisbenzimidazole. Since the emission spectra of the two fluorochromes are different, the labelled nuclei can be distinguished by the colour of their fluorescence and the numbers of trophectoderm and ICM cells identified.

Differentially labelled nuclei were mounted in glycerol, partially disaggregated and counted under fluorescence microscopy. Normal nuclei had a distinct nuclear outline, brightly staining nucleoli and even shape. Cells in mitosis, with visible chromosomes, were clearly discernible and were counted as single cells. Estimates of the number of dead cells were based on the presence of degenerating nuclei characterized by discrete clusters of labelled nuclear fragments. These dead cells were not included in the overall total of cell numbers. Mitotic and dead cell indices were calculated as follows:

Mitotic cell index = (no. of metaphases/total No. of cells)×100

Dead cell index = (no. of dead cells/total no. of cells + no. of dead cells)×100.

Statistical analysis

The number of embryos that reached the blastocyst stage was compared using χ^2 -analysis with Yates' correction. Differences in the distribution of total cell number and the numbers of TE and ICM cells in blastocysts, substrate uptake and production measurements, and distribution of the grade of embryos between the two groups were compared with the use of Wilcoxon rank sum (Mann–Whitney) test. Analysis was performed using the Statistical Package for the Social Sciences 7.0 for Windows 95 (Microsoft Inc., Redmond, WA, USA).

Results

Embryo development in the presence of taurine or glutamine

Seventy-four normally fertilized day 2 embryos donated by 13 patients, at the 2–4-cell stage, were randomly distributed between the two media. Overall, a total of 41 (54.4%) reached the blastocyst stage by day 6 post-insemination. Thirty-six out of 41 (88%) embryos had already reached the blastocyst stage by day 5. The remainder arrested at all stages between the 5-cell stage and the morula stage.

A similar proportion of embryos developed to the morula and the blastocyst stages in the two media; 69.4% of the embryos reached the morula stage with taurine compared to 65.8% with glutamine, and 58.3% of the embryos reached the blastocyst stage in the presence of taurine compared to 52.6% in the presence of glutamine (Figure 2). The proportion of blastocysts that reached that stage by day 5 was similar for glutamine and taurine, 85% (17/20) and 90% (19/21), respectively.

The total cell number was ascertained for 35 blastocysts on day 6. The distribution of total cell numbers was similar for day 6 blastocysts cultured with taurine or glutamine, with mean \pm SEM cell numbers of 73.6 ± 6.7 and 74.2 ± 6.1 , respectively. Eighty per cent of the blastocysts had more than 50 cells. No difference was observed for the dead cell indices, 9.8 ± 1.8 and

8.6 ± 1.2 (SEM) in the presence of taurine and glutamine respectively.

Seventeen blastocysts with taurine and 18 with glutamine were successfully differentially labelled on day 6 (Table I). The numbers of trophoblast cells and ICM cells in blastocysts were similar in presence of taurine or glutamine (Table I). There was no significant difference in the percentage of cells in mitosis or cells undergoing cell death in day 6 blastocysts with taurine or glutamine (Table I).

Pyruvate uptake by embryos cultured in the presence of taurine or glutamine was similar throughout development, as was the lactate production after the 8-cell stage (Figure 3). Before this stage, lactate production was 4-fold higher in the presence of taurine ($P < 0.01$).

Embryo development in the presence of glutamine with or without taurine

Seventy-six normally fertilized embryos at the 2–4-cell stage and unsuitable for transfer or freezing were donated by 23 patients. They were randomly distributed between two media, glucose-free EBSS supplemented with 1 mmol/l glutamine with or without 5 mmol/l taurine. Overall, a total of 42 (55.3%) reached the blastocyst stage by day 6 post-insemination. Twenty-nine (69%) blastocysts had already cavitated by day 5.

Supplementation of glucose-free EBSS with 1 mmol/l glutamine in combination with 5 mmol/l taurine had no significant effect on the proportion of embryos developing to the morula or blastocyst stage; 65% of the embryos reached the morula stage in the presence of glutamine compared to 52.8% in the presence of glutamine and taurine, and 62.5% of the embryos reached the blastocyst stage with glutamine compared to 47.2% with glutamine and taurine (Figure 4).

The total cell number was determined for 35 blastocysts on day 6. The mean ± SEM cell number was similar for blastocysts cultured with glutamine solely or in combination with taurine, 63.0 ± 4.6 compared to 61.0 ± 5.1, respectively. No differences were observed for the percentage of cells in mitosis or

undergoing cell death in blastocysts cultured with glutamine or with glutamine and taurine. Dead indices were 11.7 ± 2.2 and 9.2 ± 1.8 with glutamine alone or in combination with taurine respectively.

Seventeen blastocysts with glutamine and eleven with glutamine and taurine were successfully differentially labelled. Due to incomplete dissolution of the zona pellucida, the remaining blastocysts were incompletely differentially labelled with some of the trophoctoderm nuclei being labelled just with bisbenzimidazole. However, in these embryos it was possible to determine the total cell count. No differences in the distribution of trophoctoderm or ICM cell numbers were observed in blastocysts cultured with glutamine compared to blastocysts with glutamine and taurine (Table II). There was no difference in the percentage of dead cells in the trophoctoderm lineage in the presence of glutamine with or without taurine (Table II). The number of ICM cells undergoing cell death with glutamine and taurine was half that with only glutamine, although not statistically significant (Table II).

For technical reasons, no metabolic measurements could be performed on the culture media for this experiment.

Discussion

This study reports for the first time the effect of taurine on human preimplantation embryo development *in vitro* from the 2–4-cell stage to the blastocyst stage. EBSS supplemented with taurine supports blastocyst development to the same extent as EBSS supplemented with glutamine. Total cell numbers were similar for the two groups (Table I). However, taurine did not further increase the beneficial effects of glutamine which have been recently described ([Devreker *et al.*, 1998](#)); blastocyst development and cell numbers were similar for embryos cultured with glutamine in the presence or absence of taurine.

Fluids from the female reproductive tract in various mammalian species, including human, contain energy substrates, proteins and amino acids in concentrations different from those usually present in media used for the

culture of preimplantation embryos (Gardner and Leese, 1990). Taurine is present in high concentrations in mouse oviduct (Dumoulin *et al.*, 1992b) and human uterine fluids (Casslèn, 1987). Its concentration during the human menstrual cycle varies from 3 to 17 mmol/l, reaching a maximum during the luteal phase. This is 400-fold higher than plasma concentrations. The fact that taurine may play role in preimplantation embryo development is supported by the high intracellular concentrations and the presence of cellular transport mechanisms found in mouse embryos (Van Winkle *et al.*, 1994; Van Winkle and Dickinson, 1995). Indeed, mouse embryos lose between 13% and 38% of their intracellular content of taurine when cultured for 24 h in taurine-free medium (Van Winkle and Dickinson, 1995; Dumoulin *et al.*, 1997). Furthermore, medium supplemented with taurine promotes embryo development to the blastocyst stage of several species including mouse (Dumoulin *et al.*, 1992a,b), cow (Liu and Foote, 1995), hamster (Bavister and McKiernan, 1993), pig (Reed *et al.*, 1992) and rabbit (Li *et al.*, 1993).

It has been proposed that taurine may have a number of different mechanisms of action including osmolyte regulation, modulation of calcium levels and membrane stabilization (Huxtable, 1992; Timbrell *et al.*, 1995). Embryos cultured in hypo-osmotic medium release taurine into the extracellular environment whereas in hyperosmotic conditions the intracellular content of taurine remains stable (Dumoulin *et al.*, 1997). Taurine was found to protect the embryo from high potassium concentrations; the impairment of mouse embryo development in the presence of high potassium concentrations can be reversed if taurine is added to the culture medium (Dumoulin *et al.*, 1992b). Taurine also preserves sperm motility and fertilizing ability in the presence of high potassium concentrations (Mrsny and Meizel, 1985). This action is mediated through the inhibition of the $\text{Na}^+ - \text{K}^+$ ATPase enzyme, a plasma membrane enzyme that controls the intracellular content of potassium. Both taurine and potassium are found in high concentrations in oviduct and uterine fluid. The potassium content varied with the stage of the menstrual cycle, and was found to be as high as 9.9 mmol/l in the oviduct and 21 mmol/l in human uterine fluid (Lippes *et al.*, 1972; Borland *et al.*, 1980;

[Casslèn, 1984](#)). High levels of taurine in reproductive tract fluid could protect both sperm and embryos against high potassium concentrations. The low potassium concentrations in the culture medium used in this study (5.37 mmol/l) may explain why taurine does not increase blastocyst formation rates and cell numbers above those seen in the presence of glutamine. Furthermore, it has been suggested that glutamine itself could possibly act as an osmolyte ([Lawitts and Biggers, 1992](#)).

The overall blastocyst percentage achieved in this study (57.7%) in glucose-free EBSS with glutamine is lower than that reported in a previous study from one of these laboratories using identical culture conditions (71%; [Devreker et al., 1998](#)) but is higher than previously reported for media supplemented with glucose (42%, [Hardy et al., 1989a](#); 48%, [Conaghan et al., 1993](#); 41%, [Hardy et al., 1995](#)). This is probably due to the lower quality of the embryos included in this study. Previously all untransferred embryos from each patient were included in the study ([Devreker et al., 1998](#)). In the second part of this study, only embryos that were considered unsuitable for either transfer or cryopreservation on day 2 after insemination were included. Indeed, significantly more embryos of grade III were included in the second experiment (Figure 1). It has been observed that minor fragmentation does not compromise embryo development. It is only when one blastomere is completely fragmented that embryo development is significantly compromised (K.Hardy, unpublished data). Blastocyst cell numbers and the allocation of cells to the trophoctoderm and ICM are similar to a previous report using glucose-free medium supplemented with glutamine ([Devreker et al., 1998](#)). The difference in the total cell numbers for day 6 blastocysts between the two experiments can also be explained by the difference in the quality of the embryos included in the two parts of the study. Blastocyst morphology is undoubtedly related to cell number ([Hardy et al., 1989a](#)) and the proportion of embryos of lower grade was higher in the second part of the study.

Pyruvate uptake and lactate release were measured to assess a possible influence of taurine on embryo metabolism. Pyruvate uptake has been related to embryo viability with embryos that

develop to the blastocyst stage having a higher pyruvate uptake compared to those that arrest development ([Hardy *et al.*, 1989b](#)). On the other hand, high levels of lactate production could reflect embryos with reduced viability. Pyruvate uptake was similar in the presence of glutamine or taurine. The reason for the high lactate production before the 8-cell stage in the presence of taurine is unclear. This observation highlights the suggestion that taurine, as in the mouse, acts mainly during early cleavage.

Supplementation of the culture medium with taurine alone supported blastocyst development equivalent to that observed in culture medium supplemented with glutamine. However, taurine did not further potentiate the beneficial effects of glutamine ([Devreker *et al.*, 1998](#)). Studies in the mouse show that taurine is only beneficial during the first 48 h of culture ([Dumoulin *et al.*, 1992a](#)), at the time of genome activation. However, it did not help to overcome the 2-cell block observed with outbred mice ([Dumoulin *et al.*, 1992a](#)). In this study, the supernumerary embryos were only available for culture 45 h post-insemination, which may also be after the time when human embryos could be responsive to taurine. Here, no difference was observed in the proportion of embryos that arrest development between the 4- and 8-cell stages (when embryonic genome activation is believed to occur in humans) for the different culture media (Figures 2 and 4).

Here we have demonstrated that taurine supports human embryo development to the blastocyst stage *in vitro*. However, taurine did not enhance further the beneficial effects of glutamine. Medium supplementation with taurine could prevent the depletion of the intracellular content of the preimplantation embryos during the time of culture, allowing them to preserve their osmoregulatory mechanisms during preimplantation development *in vitro*. However, to confirm a specific effect of taurine, one should examine embryo development when taurine is supplied during the first 48 h after fertilization, in contrast to the present study where taurine was added 45 h after fertilization.

Table I. Number of trophectoderm and inner cell mass cells in day 6 blastocysts in the presence of 1 mmol/l glutamine or 5 mmol/l taurine

Amino acids	n	Trophectoderm			Inner cell mass		
		Cell number (range)	Mitotic index	Dead index	Cell number (range)	Mitotic index	Dead index
Glutamine	18	51.5 ± 5.5 (22–109)	0.6 ± 0.3	8.1 ± 1.6	22.7 ± 2.5 (8–40)	0 ± 0	1
Taurine	17	53.6 ± 6.0 (24–106)	0.3 ± 0.2	8.9 ± 2.2	21.3 ± 2.9 (11–42)	0.6 ± 0.6	1

Values are mean ± SEM. Values are not statistically significantly different.

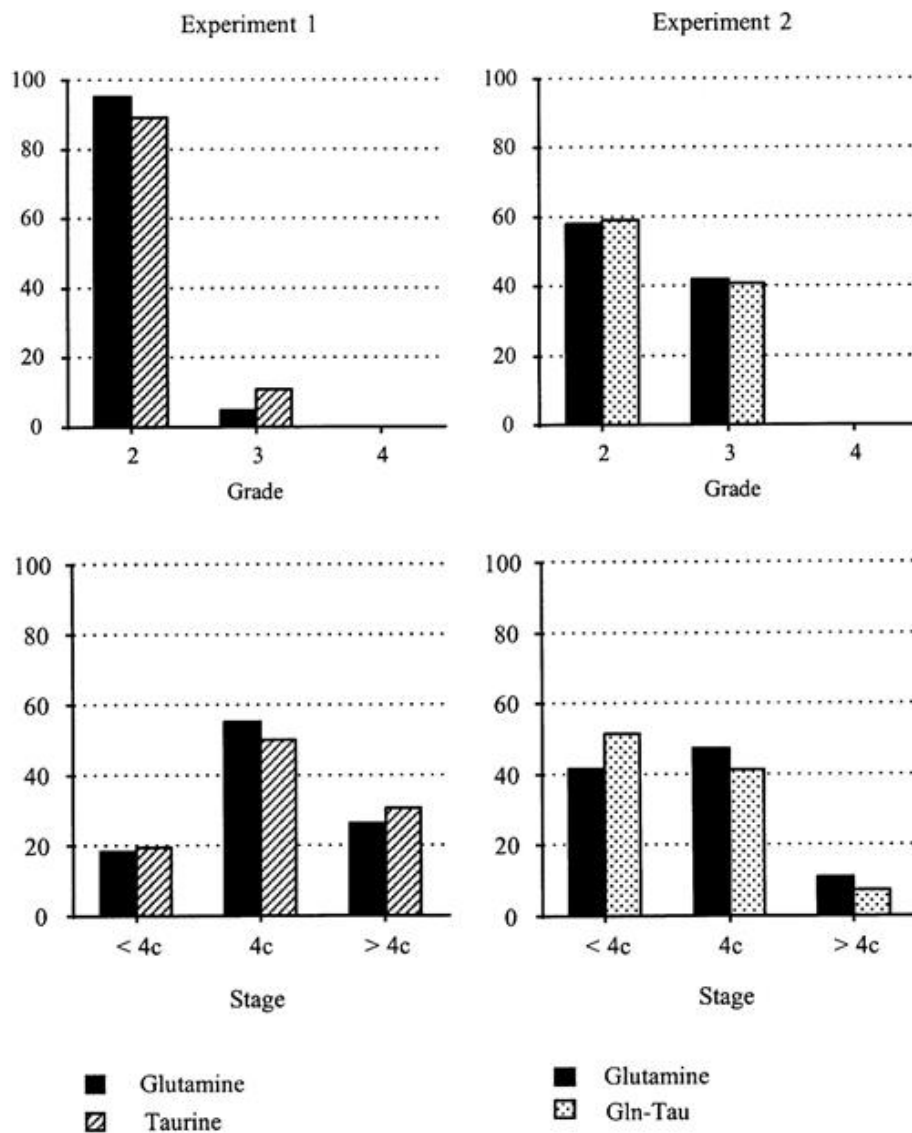
One blastocyst with glutamine and two with taurine were excluded from the analysis because of the presence of a majority of dead cells. One blastocyst with glutamine and two with taurine were lost during labelling.

**Table II.** Number of trophectoderm and inner cell mass cells in day 6 blastocysts in the presence of 1 mmol/l glutamine with or without 5 mmol/l taurine

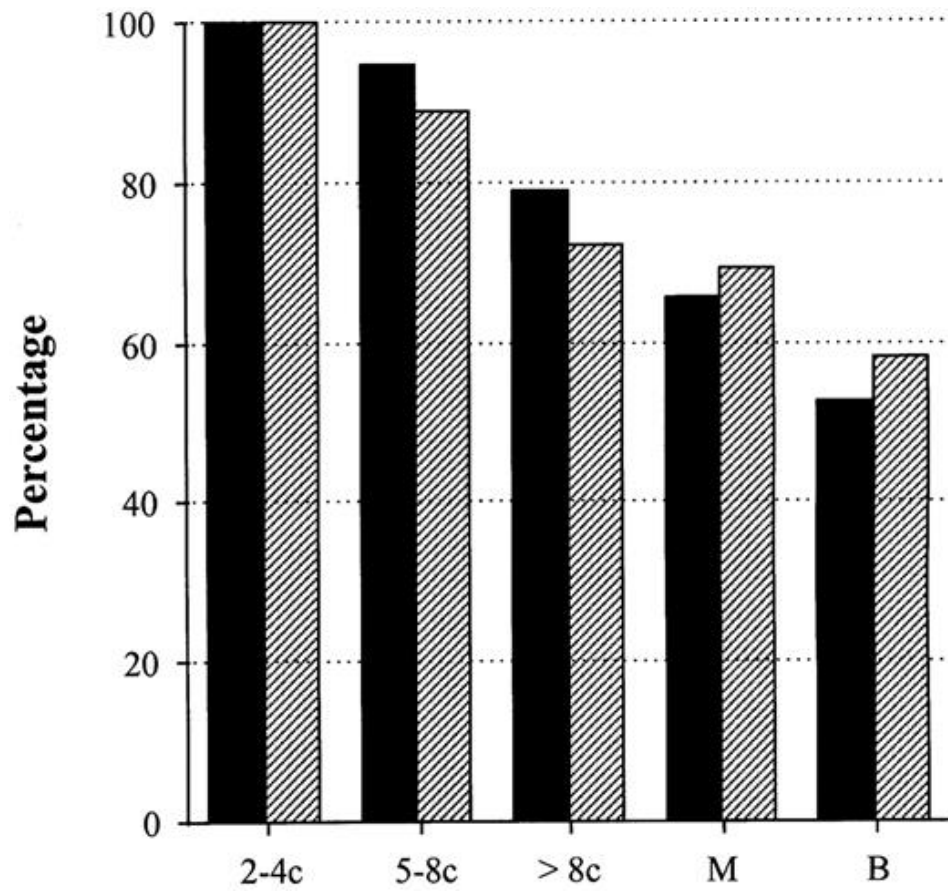
Amino acids	n	Trophectoderm			Inner cell mass		
		Cell number (range)	Mitotic index	Dead index	Cell number (range)	Mitotic index	Dead index
Glutamine	17	45.8 ± 3.9 (26–92)	1.0 ± 0.4	11.0 ± 1.9	20.7 ± 2.1 (9–35)	0.4 ± 0.4	1
Glutamine and taurine	11	41.2 ± 4.3 (26–79)	1.6 ± 1.1	9.7 ± 1.9	17.9 ± 2.0 (11–30)	0	5

Values are mean ± SEM. Values are not statistically different.

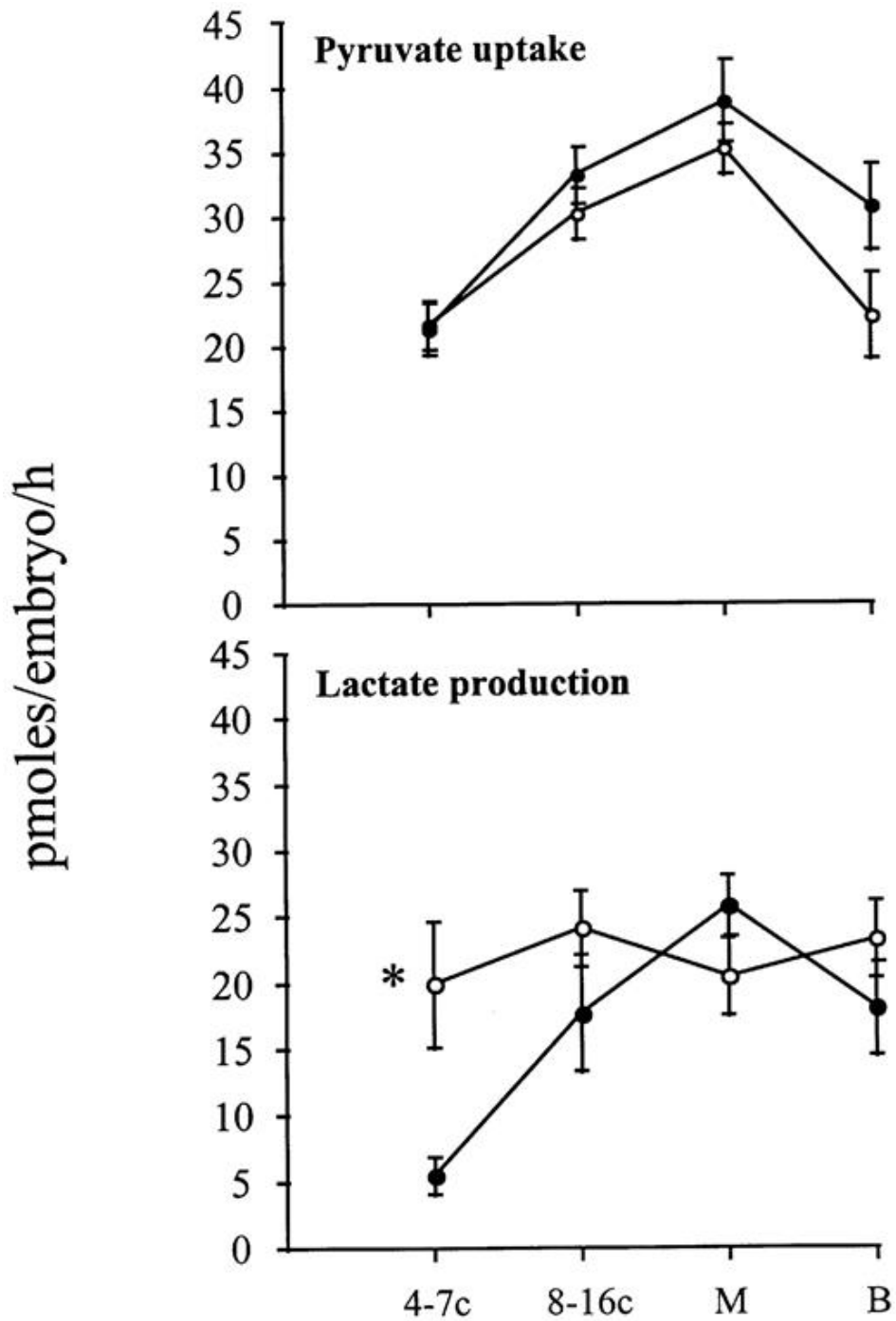


Figure 1.

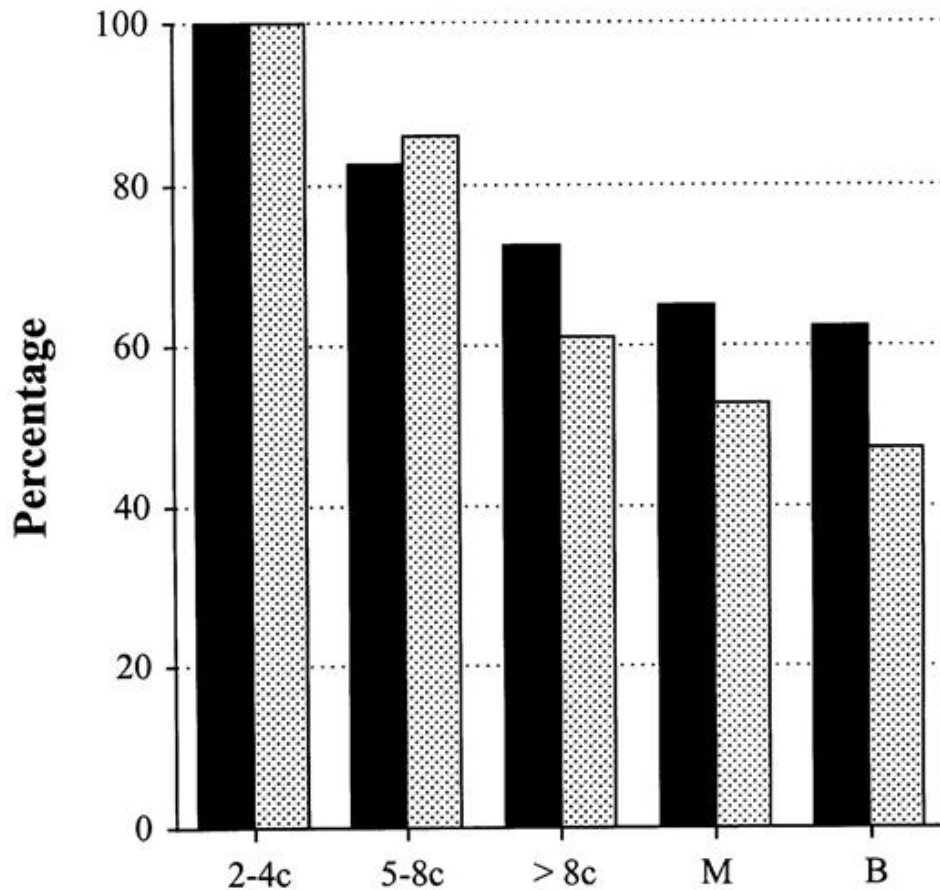
Percentage distribution of grades of embryos (upper panels) and of cell number (lower panels) for embryos allocated to each set of culture conditions in experiment 1 (■ 1 mmol/l glutamine alone, ▨ 5 mmol/l taurine alone) and experiment 2 (■ 1 mmol/l glutamine alone, ▤ 1 mmol/l glutamine and 5 mmol/l taurine). The distribution of grades is significantly different between experiment 1 and 2 ($P < 0.01$).

Figure 2.

Percentage of embryos reaching the blastocyst stage with glutamine (■, $n = 38$) or taurine (▨, $n = 36$). M = morula, B = blastocyst.

Figure 3.

Pyruvate uptake and lactate production by embryos that reached the blastocyst stage with glutamine (•, $n = 38$) or with taurine (○, $n = 36$). Values are mean \pm SEM ($*P < 0.01$).

Figure 4.

Percentage of embryos reaching the blastocyst stage with glutamine (■, $n = 40$) or with glutamine and taurine (▨, $n = 36$). M = morula, B = blastocyst.

4 To whom correspondence should be addressed

The work was supported by a grant from the 'Fondation Erasme', Free University of Brussels, Belgium.

References

Bavister, B.D. (1995) Culture of preimplantation embryos: facts and artifacts. *Hum. Reprod. Update*, 1, 91–148.

Bavister, B.D. and Arlotto, T.M. (1990) Influence of single amino acids on the development of hamster 1-cell embryos *in vitro*. *Mol. Reprod. Dev.*, 25, 45–51.

Bavister, B.D. and McKiernan, S.H. (1993) Regulation of hamster embryo development *in vitro* by amino acids. In Bavister, B. (ed.), *Preimplantation Embryo Development*. Springer-Verlag, New York, 57 pp.

Borland, R.M., Biggers, J.D. and Lechene, C.P. (1980) Elemental composition of fluid in the human fallopian tube. *J. Reprod. Fertil.*, 58, 479–482.

Carney, E.W. and Bavister, B.D. (1987) Stimulatory and inhibitory effects of amino acids on development of hamster eight-cell embryos *in vitro*. *J. In Vitro Fertil. Embryo Transfer*, 4, 162–167.

Casslèn, B.G. (1984) Human uterine fluid, examined in undiluted samples for osmolarity and the concentrations of inorganic ions, albumin, glucose and urea. *Am. J. Obstet. Gynecol.*, 150, 877–881.

Casslèn, B.G. (1987) Free amino acids in human uterine fluid. Possible role of high taurine concentration. *J. Reprod. Med.*, 32, 181–184.

Chatot, C.L., Ziomek, C.A., Bavister, B.D. *et al.* (1989) An improved culture medium supports development of one-cell random-bred mouse embryos *in vitro*. *J. Reprod. Fertil.*, 86, 679–688.

Chatot, C.L., Lewis, J.L., Torres, I. and Ziomek, C.A. (1990) Development of 1-cell embryos from different strain of mice in CZB medium. *Biol. Reprod.*, 42, 432–440.

Conaghan, J., Handyside, A.H., Winston, R.M.L. and Leese, H.J. (1993) Effects of pyruvate and glucose on the development of human preimplantation embryos *in vitro*. *J. Reprod. Fertil.*, 99, 87–95.

Dawson, K.J., Conaghan, J., Ostera, G.R. *et al.* (1995) Delaying transfer to the third day post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. *Hum. Reprod.*, 10, 177–182.

Devreker, F., Govaerts, I., Bertrand, E. *et al.* (1996) The long-acting gonadotropin-releasing hormone analogues impaired the implantation rate. *Fertil. Steril.*, 65, 122–126.

Devreker, F., Winston, R.M.L. and Hardy, K. (1998) Glutamine improves human preimplantation development *in vitro*. *Fertil. Steril.*, 69, 293–299.

Dumoulin, J.C.M., Evers, J.L.H., Bakker, J.A. *et al.* (1992a) Temporal effects of taurine on mouse preimplantation development *in vitro*. *Hum. Reprod.*, 7, 403–407.

Dumoulin, J.C.M., Evers, J.L.H., Bras, M. *et al.* (1992b) Positive effect of taurine on preimplantation development of mouse embryos *in vitro*. *J. Reprod. Fertil.*, 94, 373–380.

Dumoulin, J.C.M., Van Wissen, L.C.P., Menheere, P.P.C.A. *et al.* (1997) Taurine acts as an osmolyte in human and mouse oocytes and embryos. *Biol. Reprod.*, 56, 739–744.

Gardner, D.K. and Lane, M. (1993) Amino acids and ammonium regulate mouse embryo development in culture. *Biol. Reprod.*, 48, 377–385.

Gardner, D.K. and Lane, M. (1997) Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Hum. Reprod. Update*, 3, 367–382.

Gardner, D.K. and Leese, H.J. (1990) Concentrations of nutrients in mouse oviduct fluid and their effects on embryo development and metabolism. *J. Reprod. Fertil.*, 88, 361–368.

Gardner, D.K., Lane, M., Kouridakis, K. and Schoolcraft, W.B. (1997) Complex physiologically based serum-free culture media increase mammalian embryo development. In Gomel, V. and Leung, P.C.K. (eds), *In Vitro Fertilization and Assisted Reproduction*. Monduzzi Editore, Bologna, Italy, pp. 187–191.

Gwatkin, R.B. and Haidi, A.A. (1973) Requirements for the maturation of hamster oocytes *in vitro*. *Exp. Cell Res.*, 76, 1–7.

Hardy, K. (1993) Development of human blastocysts *in vitro*. In Bavister, B. (ed.), *Preimplantation Embryo Development*. Springer-Verlag, New York, pp. 184–199.

Hardy, K., Handyside, A.H. and Winston, R.M.L. (1989a) The human blastocyst: cell number, death and allocation during late preimplantation development *in vitro*. *Development*, 107, 597–604.

Hardy, K., Hooper, M.A.K., Handyside, A.H. *et al.* (1989b) Non-invasive measurement of glucose and pyruvate uptake by individual human oocytes and preimplantation embryos. *Hum. Reprod.*, 4, 188–191.

Hardy, K., Robinson, F.M., Paraschos, T. *et al.* (1995) Normal development and metabolic activity of preimplantation embryos *in vitro* from patients with polycystic ovaries. *Hum. Reprod.*, 10, 2125–2135.

Hervann, A., Gonzales, J., Troupel, S. and Galli, A. (1986) Amino acid content of human semen in normal and infertility cases. *Andrologia*, 18, 461–469.

Hillier, S.G., Dawson, K.J., Afnan, M. *et al.* (1984) Embryo culture quality: quality control in *in vitro* fertilization. In Thompson, W., Joyce, D.N. and Newton, J.R. (eds), *Proceedings of the Twelfth Study Group of the Royal College of Obstetricians and Gynecologists Royal College of Obstetricians and Gynecologists*, London, 125 pp.

Huxtable, R.J. (1992) Physiological actions of taurine. *Physiol. Rev.*, 72, 101–163.

Kane, M.T. and Foote, R.H. (1970) Culture of two-and four -cell rabbit embryos to the expanding blastocyst stage in synthetic media. *Proc. Soc. Exp. Biol. Med.*, 113, 921–925.

Kishi, J., Noda, Y., Narimoto, K. *et al.* (1991) Block to development in cultured rat one-cell embryos is overcome using medium HECM-1. *Hum. Reprod.*, 6, 1445–1448.

Kola, I., Sathanathan, A.H. and Gras, L. (1993) Chromosomal analysis of preimplantation mammalian embryos. In Trounson, A. and Gardner, D.K. (eds), *Handbook of In Vitro Fertilization*. CRC Press, Boca Raton, FL, 173 pp.

Lane, M. and Gardner, D.K. (1994) Increase in postimplantation development of cultured mouse embryos by amino acids and induction of fetal retardation and exencephaly by ammonium ions. *J. Reprod. Fertil.*, 102, 305–312.

Lane, M. and Gardner, D.K. (1997) Differential regulation of mouse embryo developments and viability by amino acids. *J. Reprod. Fertil.*, 109, 153–164.

Lawitts, J.A. and Biggers, J.D. (1992) Joint effects of sodium chloride, glutamine and glucose in mouse preimplantation embryo culture media. *Mol. Reprod. Dev.*, 31, 189–194.

Leese, H.J. and Barton, A.M. (1984) Pyruvate and glucose uptake by mouse ova and preimplantation embryos. *J. Reprod. Fertil.*, 72, 9–13.

Leese, H.J., Aldridge, S. and Jeffries, K.S. (1979) The movement of amino acids into rabbit fluid. *J. Reprod. Fertil.*, 56, 623–626.

Li, J., Foote, R.H. and Simkin, M. (1993) Development of rabbit zygotes cultured in protein-free medium with catalase, taurine, or superoxide dismutase. *Biol. Reprod.*, 48, 33–37.

Lippes, J., Enders, R.G., Pragay, D.A. and Bartholomew, W.R. (1972) The collection and analysis of human Fallopian tubal fluid. *Contraception*, 5, 85–94.

Liu, Z. and Foote, R.H. (1995) Development of bovine embryos in Ksom with added superoxide dismutase and taurine and five and twenty percent O₂. *Biol. Reprod.*, 53, 786–790.

Miller, J.G.O. and Schultz, G.A. (1987) Amino acid content of preimplantation rabbit embryos and fluids of the reproductive tract. *Biol. Reprod.*, 36, 125–129.

Mrsny, R.J. and Meizel, S. (1985) Inhibition of hamster Na⁺, K⁺-ATPase activity by taurine and hypotaurine. *Life Sci.*, 36, 271–275.

Mrsny, R.J., Waxman, L. and Meizel, S. (1979) Taurine maintains and stimulates motility of hamster sperm during capacitation *in vitro*. *J. Exp. Zool.*, 210, 123–128.

Nasr-Esfahani, M.H., Winston, N.J. and Johnson, M.H. (1992) Effects of glucose, glutamine, ethylenediaminetetraacetic acid and oxygen tension on the concentration of reactive oxygen species and development of the mouse preimplantation embryo *in vitro*. *J. Reprod. Fertil.*, 96, 219–231.

Reed, M.L., Jin, D.I. and Petters, R.M. (1992) Glucose and inorganic phosphate inhibits rat 8-cell embryo development *in vitro*. *Theriogenology*, 37, 282.

Schultz, G.A., Kaye, P.L., McKay, D.J. and Johnson, M.H. (1981) Endogenous amino acid pool sizes in mouse eggs and preimplantation embryos. *J. Reprod. Fertil.*, 61, 387–393.

Tesarik, J. (1994) Developmental failure during the preimplantation period of human embryogenesis. In Van Blerkom, J. (ed.), *The Biological Basis of Early Human Reproductive Failure*. Oxford University Press, New York, 327 pp.

Timbrell, J.A., Seabra, V. and Waterfield, C.J. (1995) The *in vivo* and *in vitro* protective properties of taurine. *Gen. Pharmacol.*, 26, 453–462.

Van Winkle, L.J. and Dickinson, H.R. (1995) Differences in amino acid content of preimplantation mouse embryos that develop *in vitro* versus *in vivo*: *In vitro* effects of five amino acids that are abundant in oviductal secretions. *Biol. Reprod.*, 52, 96–104.

Van Winkle, L.J., Wasserlauf, H.G., Dickinson, H.R. and Campione, A.L. (1994) Osmotic regulation of taurine transport via system B and novel processes in mouse preimplantation conceptuses. *Biochim. Biophys. Acta*, 1191, 244–255.

Velázquez, A., Delgado, N.M. and Rosado, A. (1986) Taurine content and amino acid composition of human acrosome. *Life Sci.*, 38, 991–995.

Walker, S.K., Hill, J.L., Kleeman, D.O. and Nancarrow, C.D. (1996) Development of ovine embryos in synthetic oviductal fluid containing amino acids at oviductal concentrations. *Biol. Reprod.*, 55, 703–708.

© European Society of Human Reproduction and Embryology