Effects of taurine on human embryo development
\textit{in vitro}

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Glutamine and taurine are reported to be beneficial for mouse embryo development \textit{in vitro}, and we have recently shown that glutamine improves human blastocyst formation \textit{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 \pm 4.6 and 61.0 \pm 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.

\textit{Key words:} embryo metabolism/glutamine/human blastocyst/taurine

Introduction

Despite improvements in IVF, implantation rates remain disappointingly low. Only \textasciitilde{}20\% of transferred embryos implant (Dawson et al., 1995). In addition, \textasciitilde{}50\% of normally fertilized embryos cultured \textit{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 \textit{in vitro}. Female reproductive tract fluids contain high concentrations of certain amino acids such as taurine, glutamate, glutamate or glycine (Leese et al., 1979; Borland et al., 1980; Casslén 1987; Miller and Schulz 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 \textit{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 \textit{in vitro}, with blastocysts having a potential almost equivalent to those developed \textit{in vivo} (Lane and Gardner, 1997). Few studies have addressed the effects of specific amino acids on human embryo development \textit{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 \textit{in vitro} (Devreker et al., 1998).

Taurine is a \(\beta\)-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 (Casslén, 1987). Taurine is also present in human semen (Hernvann 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 (Mirsny 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 pre-implantation 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 16 h 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.

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 (Casslén, 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 µl 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 µl aliquots of the spent and control drops of medium from the recently terminated culture were diluted with 398 µl of a 5 µmol/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.
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 ($P < 0.01$).

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, bisbenzimide. 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 $\chi^2$-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 ± 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 trophectoderm 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

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**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 ± SEM (*$P < 0.01$).

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>n</th>
<th>Trophoderm</th>
<th>Inner cell mass</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cell number (range)</td>
<td>Mitotic index</td>
</tr>
<tr>
<td>Glutamine</td>
<td>18</td>
<td>51.5 ± 5.5 (22–109)</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Taurine</td>
<td>17</td>
<td>53.6 ± 6.0 (24–106)</td>
<td>0.3 ± 0.2</td>
</tr>
</tbody>
</table>

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.
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 trophectoderm nuclei being labelled just with bisbenzimide. However, in these embryos it was possible to determine the total cell count. No differences in the distribution of trophectoderm 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 trophectoderm 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 (Casslén, 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 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 (Huxttable, 1992; Timbrell et al., 1995). Embryos cultured in hypo-osmotic medium release taurine into the extracellular environment whereas in hyperosmotic conditions the intra-

### 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

<table>
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<tr>
<td></td>
<td></td>
<td>Cell number (range)</td>
<td>Mitotic index</td>
</tr>
<tr>
<td>Glutamine</td>
<td>17</td>
<td>45.8 ± 3.9 (26–92)</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Glutamine and taurine</td>
<td>11</td>
<td>41.2 ± 4.3 (26–79)</td>
<td>1.6 ± 1.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. Values are not statistically different.
cellular 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 Na$^{+}$-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 (Lrippes et al., 1972; Borland et al., 1980; Casslén, 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. 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 trophoderm 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.

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References


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